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

VICTOR VIAU AND MICHAEL J. MEANEY

Developmental Neuroendocrinology Laboratory, Douglas Hospital-McGill University Research Institute, Departments of Psychiatry, and Neurology and Neurosurgery, Faculty of Medecine, McGill University, Montréal, Canada H4H 1R3

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 (E_2) and progesterone (P). In cycling rats, we found significantly higher peak ACTH ($P < 0.01$) and B ($P < 0.05$) responses to stress during proestrus compared to the estrous and diestrous phases. No differences were found in either basal ACTH and B levels across the cycle phases. In a separate study, OVX rats were maintained on low, physiological levels of E_2 and P with silastic implants for 3 days, and injected either with oil (O′), 10 μg of E_2 (E′) 24 h before stress testing, or with E_2 and 500 μg P 24 and 4 h, respectively, prior to stress (EP′). These treatments mimicked endogenous profiles of E_2 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 after 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. β-endorphin-like immunoreactive 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. In contrast to the effect seen at 24 h, ACTH responses to stress 48 h after E_2 injection in the E′ group were comparable to O′ animals. There was no effect of E_2 on ACTH clearance, whereas B clearance was enhanced in E′ treated animals *vs.* O′-treated animals. 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 activational and inhibitory components of HPA activity. (*Endocrinology* **129:** 2503–2511, 1991)

STRESS inhibits reproductive function, and this effect has been observed as a decrease in gonadotrophin secretion (1, 2), as well as an inhibition of sexual behavior (3, 4). Gonadal steroids in turn, are known to affect hypothalamic-pituitary-adrenal (HPA) function and HPA activity under both basal and stressful conditions. Female rats typically show greater adrenocorticotrophin (ACTH) and corticosterone (B) responses to stress (5, 6), and also secrete higher basal levels of B (7). The sex difference in ACTH and B levels during stress is abolished by ovariectomy (OVX) and is reinstated by estradiol (E_2) administration (8). Although female rats secrete higher basal levels of B, circulating levels of corticosterone-binding-globulin are also elevated, thus reducing exposure to free, bioactive B to levels comparable to those in males (9).

Basal levels of ACTH and B have been observed to increase about the time of proestrous in the rat (7). Likewise, in women during the menstrual cycle, ACTH and cortisol levels rise toward the end of the follicular phase (10). It is during ovulation when E_2 levels are highest in the rat, and in women during ovulation and the midluteal phase. During proestrous in the rat, higher B levels occur in response to stress than during diestrous and estrous (11, 12). Cortisol, as well as catecholaminergic responses to stress also vary as a function of menstrual phase (13–15). Moreover, postdexamethasone cortisol values are higher in subjects tested during the middle 2 weeks of the menstrual cycle (about the time of ovulation), compared to the other weeks of the cycle (16). 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 cortisol secretion as well as blunted responses to corticotropin-releasing hormone (CRH) (17). Likewise, female rats lacking circadian B rhythms, show irregular ovulatory cycles (18).

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Address all correspondence and requests for reprints to: Michael J. Meaney, Douglas Hospital Research Centre, 6875 Boulevard LaSalle, Montreal (Quebec) H4H 1R3, Canada.

E_2 receptors are localized in brain regions that mediate HPA function (19, 20) and E_2 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 (21), oxytocin (OT) and arginine vasopressin (AVP) (22, 23), corticotroph ACTH synthesis and secretion (24), and glucocorticoid metabolism (25). *In vivo,* OVX decreases pituitary synthesis and release of ACTH as well as adrenal synthesis of B (24, 26). These effects are reversible by E_2 replacement, consistent with increased ACTH release and adrenal secretion of B (26, 27). In contrast, corticotrophin-releasing activity in median eminence extracts is not affected by OVX, but chronic administration of high levels of E_2 in OVX rats decreases releasing activity (27) and hypothalamic content of CRH (21). Likewise, chronic E_2 treatment decreases the adrenal secretory capacity of B (27). However, the ACTH response of pituitaries incubated with uniform CRH stimulation is depressed in OVX animals, and this effect is partially reversible by E_2 administration (27).

Taken together, these somewhat conflicting data show that gonadal steroids can regulate several aspects of HPA function. At this point, however, it is not clear whether these actions result in changes in HPA function in response to dynamic variations in circulating estrogen and/or progesterone levels. The data emerge from experiments using prepuberal rats, chronic E_2 treatments, and OVX animals without replacement. The pertinence of these findings in understanding the effects of dynamic variations in E_2 levels that occur during the estrous cycle on HPA responsivity to stress is not clear. Moreover, studies investigating whether variations in HPA response to stress occur over the estrous cycle, have examined only adrenal activity by measuring circulating levels of B (11, 12). Since peak B responses are at or near maximal levels at low stress intensities (28) and gonadal steroids are known to regulate adrenal function, measures of B alone may not adequately reflect changes in the level of hypothalamic activity.

To further examine the relationship between HPA activity and the estrous cycle we have examined the plasma ACTH and B responses to 20 min of restraint stress in cycling and in OVX rats treated acutely with estrogen and progesterone in a manner that approximates the variations observed over the estrous cycle.

Materials and Methods

The animals used were female Long-Evans, hooded rats (Charles River Canada, St. Constant, Quebec, Canada) weighing approximately 225–250 g 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, 0.025 ID, 0.047 OD) 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. Blood samples were collected in iced tubes containing EDTA and Trasylol (Aprotonin; Miles Canada Inc.), centrifuged at 3000 × *g* for 10 min, after which plasma was stored in separate aliquots at −80 C until assayed.

Plasma B was measured by RIA (29) with a highly specific B antiserum (B3-163, Endocrine Sciences, Tarzana, CA). Plasma ACTH was measured using the RIA kit of ICN-Biochemicals (Carson, CA). The ACTH antibody cross-reacts 100% with ACTH 1–39 and ACTH 1–24, but does not cross-react with β-endorphin, α- and β-MSH, α- and β-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. β-Endorphin-like immunoreactivity, was determined by RIA, using an antiserum specific for the C-terminus of β-endorphin (β-END). This β-END antibody (30) cross-reacts almost 100% with bovine β-lipotrophin (β-LPH) and α-*N*-acetylated β-END, 70% with bovine β-END_{1-27}, but does not cross-react with ACTH, α-MSH, β-MSH, nor with bovine β-LPH fragments 61–65, 62–67, and 80–84. Thus, the β-endorphin-like immunoreactivity measured includes recognition of POMC, β-LPH, both the α-*N*-acetylated and nonacetylated forms of β-END fragments 1–31 and 1–27, but no recognition for β-END fragments 1–16 and 1–17. The intra- and interassay coefficients of variation were 9.0 and 10.0%, respectively. Plasma (E_2) was measured using the RIA kit of ICN-Biochemicals. The E_2 antibody cross-reacts 100% with E_2-17β, 20% with estrone, 1.5% with estriol, and 0.7% with E_2-17α. The E_2 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). 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, E_2, 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 ml of blood sampled from individual rats at 1000 and 2200 h, during different phases of the estrous cycle. 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. Prestress 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 poststress. 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 sc with one silastic capsule (0.062 ID, 0.125 OD) containing E_2 dissolved in peanut oil (Sigma, St. Louis, MO, β-E_2 3-Benzoate, 30 μg/ml; 10 mm/100 g BW), and another capsule (0.132 ID, 0.183 OD) containing crystalline progesterone (Sigma, 4-Pregnene-3, 20, dione, 10 mm/animal). This treatment provides physiological levels of E_2 and P (31) in the range observed during diestrous (32). Forty-eight hours after receiving steroid implants, rats were then intubated with jugular catheters. Twenty-four hours later, rats received either a 0.2 ml injection of vehicle (peanut oil) sc [oil (O′) group], or an injection of 10 μg of E_2 (E′ group). Twenty hours 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 [stress (EP′) group]. Four hours 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, respectively. 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 ml) were taken immediately after the rat was placed in the restrainer (time 0), and at 5, 10, 15, and 20 min during the 20 min of restraint stress. Using the same treatment as above, we also measured the β-END/β-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 β-END/β-LPH levels are known to peak in response to restraint stress (33).

Clearance studies were performed using the O′ and E′ treated animals to determine if E_2 had acute effects on ACTH and B metabolism. In the ACTH clearance study, animals were injected with 250 μg of Dexamethasone 4 h prior to the study in order to block endogenous ACTH release. Animals were then injected iv with 0.2 ml normal saline containing 0.5 μg $ACTH_{1-39}$ and [^{125}I]$ACTH_{1-39}$ (0.2 μCi). Blood was sampled at 0.25, 0.5, 1, 2, 3, 5.5, 8, 10.5, and 13 min after ACTH administration (33). Plasma (0.1 ml) was extracted on C_{18} columns (Waters Associates, Milford MA), washed with 2 ml 60% Acetonitrile in 0.1% trifluoroacetic acid (TFA), and 5 ml 0.1% TFA, eluted with 3 ml 60% acetonitrile in 0.1% TFA, and counted to measure levels of radiolabeled ACTH. In the B clearance study, animals were injected with metyrapone (Sigma) 20 and 2 h prior to receiving B. Animals were injected sc with 0.1 ml saline:ethanol (ETOH) (9:1) solution containing 5.0 mg of B and [^{3}H]B (1.0 μCi). Blood was sampled at 15, 30, 45, 90, and 120 min after administration of CORT solution (34). Labeled B was extracted from plasma with ETOH, dried under nitrogen, resuspended in ETOH, and counted. These doses of ACTH (0.5 μg) and B (5.0 mg) were chosen to mimic the plasma levels achieved during stress.

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, prestress levels of ACTH and B did not differ across cycle phase (Fig. 1). Animals in proestrous showed significantly higher levels of ACTH ($P < 0.01$) during stress

TABLE 1. Mean (± SEM) plasma ACTH (pg/ml) and B (μg/dl) secretion under basal conditions in animals during proestrous (PRO), estrous (EST), and diestrous (DI) (n = 9, 8, 16/group, respectively).

	AM	PM
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]
B		
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]

AM, 2 h after lights on; PM, 2 h after lights off.
[a] Value that is significantly ($P < 0.05$) different from AM value.

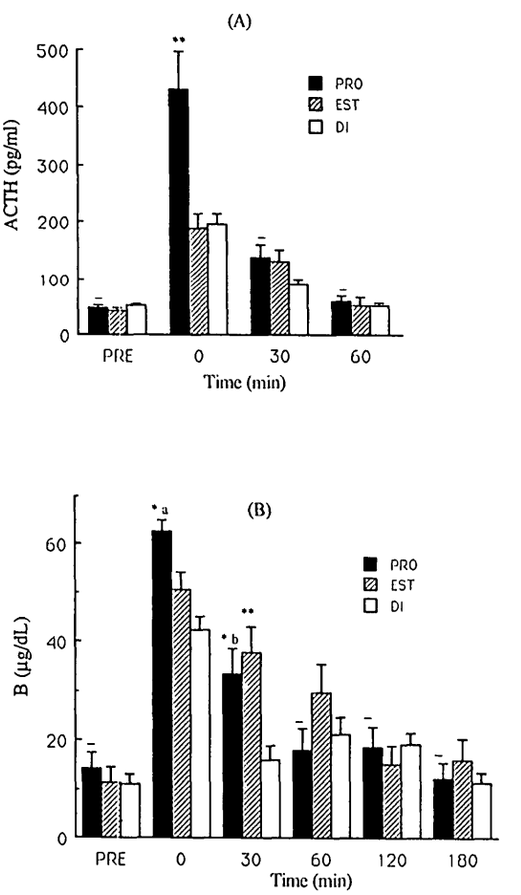


FIG. 1. Mean (±SEM) ACTH (A) and B (B) values (pg/ml, and μg/dl, respectively) of animals during proestrous (PRO), estrous (EST), and diestrous (DI), prior to (PRE) and following the termination of 20 min restraint stress (n = 13, 12, and 32 animals per phase, respectively). A, —, $P > 0.05$; **, $P < 0.01$ *vs.* EST and DI; B, —, $P > 0.05$; *a, $P < 0.05$ *vs.* EST and DI; *b, $P < 0.05$; **, $P < 0.01$ *vs.* DI.

(measured at 0 min following 20 min of restraint stress; Fig. 1A) than animals in estrous or diestrous. At 30 and 60 min poststress, 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. 1B) than animals in estrous or diestrous. At 30 min poststress, 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 poststress, 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.

HPA response to stress in OVX, steroid-treated rats

Steroid-treated rats showed the following E_2 (pg/ml) and P (ng/ml) levels (n = 5/group): O′, 71.4 ± 1.6 and 4.4 ± 0.4, respectively; E′, 510.7 ± 38.9 and 5.1 ± 0.6, respectively; EP′, 526.7 ± 95.1 and 28.9 ± 1.1, respectively. These values are within the physiological range of estrogen and progesterone levels observed during the estrous cycle of the rat (32).

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. 2A). By 30 min poststress 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 poststress (Fig. 2B). No differences were found in prestress levels of ACTH and B as a function of E_2 and P treatment. E′ animals stressed 48 h following a 10 μg dose of E_2, showed ACTH responses comparable to O′ treated animals (Fig. 3).

Within the 20-min stress period, plasma ACTH levels peaked after 10 min in all groups (Fig. 4A). 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 ± 33.5, 173.0 ± 13.6, and 178.8 ± 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. 4B). The β-END/β-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 β-END/β-LPH levels under basal conditions.

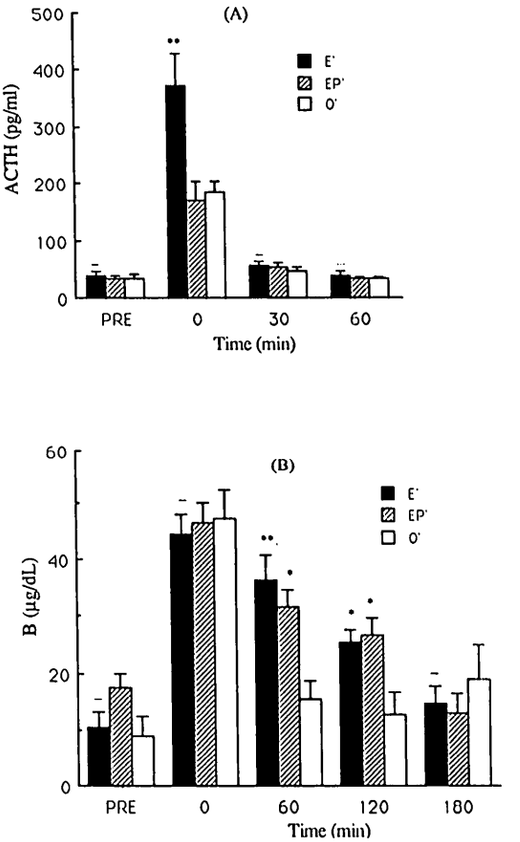


FIG. 2. Mean (±SEM) ACTH (A) and B (B) values (pg/ml and μg/dl, respectively) in E′, EP′, and O′ treated animals (see text for respective treatments), prior to (PRE) and following termination of 20 min restraint stress (n = 6, 7, and 10 animals per group, respectively). A, —, $P > 0.05$; **, $P < 0.01$ *vs.* EP′ and O′; B, —, $P > 0.05$ *vs.* EP′ and O′; *, $P < 0.05$; **, $P < 0.01$ *vs.* O′.

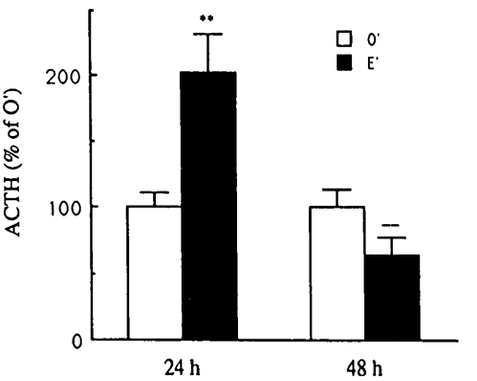
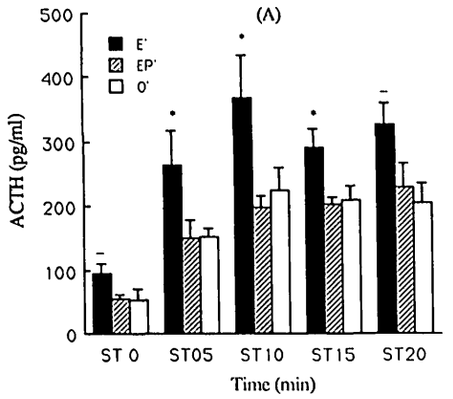


FIG. 3. Mean (±SEM) ACTH values in E′ treated animals, 24 or 48 h after E_2 (10 μg) administration (n = 7, 5, respectively), following termination of 20 min restraint stress. Values are expressed as a percentage of O′ treated animals. —, $P > 0.05$; **, $P < 0.01$ *vs.* O′.

ACTH and B clearance

Measurement of [^{125}I]ACTH and [^{3}H]B clearance rates indicated comparable half-lives in ACTH ($P > 0.05$) between the E′ and O′ treated animals (7.0 ± 0.3 and 7.3 ± 0.4 min, respectively, Fig. 5) and in B ($P > 0.05$)



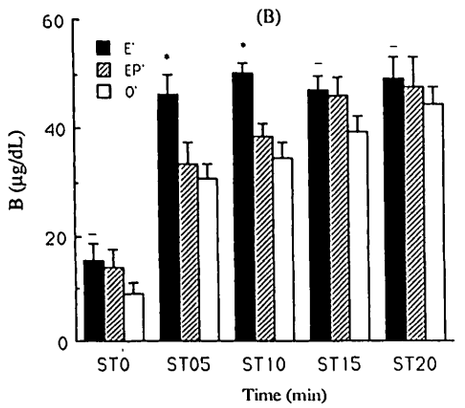


FIG. 4. Mean (±SEM) ACTH (A) and B (B) values (pg/ml and μg/dL, respectively) in E′, EP′, and O′ treated animals (see text for respective treatments), during stress (n = 6, 9, and 9 animals/group, respectively). A, —, $P > 0.05$; *, $P < 0.05$ *vs.* EP′ and O′; B, —, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$ *vs.* EP′ and O′.

TABLE 2. Mean (± SEM) plasma β-endorphin/β-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] Value significantly ($P < 0.05$) different from basal value.
[b] Differs significantly *vs.* O′ ($P < 0.01$) and EP′ ($P < 0.05$).

in the E′, EP′, and O′ treated animals (56.3 ± 5.6, 51.9 ± 2.9, and 67.2 ± 5.6 min, respectively, Fig. 6). Together, these data suggest that the differences in plasma levels of ACTH and B levels observed between groups under stress conditions cannot be explained by an effect of E_2 or P on clearance rate. The distribution volume for [^{3}H]B was approximately 5-fold higher than [^{125}I]ACTH (362.1 ± 93.9 and 74.5 ± 12.5 ml, respectively). However,

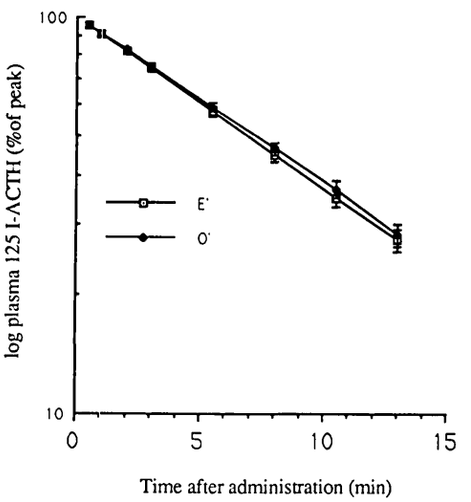


FIG. 5. Clearance of [^{125}I]ACTH$_{1-39}$ from plasma after its iv administration in OVX rats replaced with low levels of E_2 and P; O′, or with high levels of E_2 and low levels of P; E′, n = 7/group.

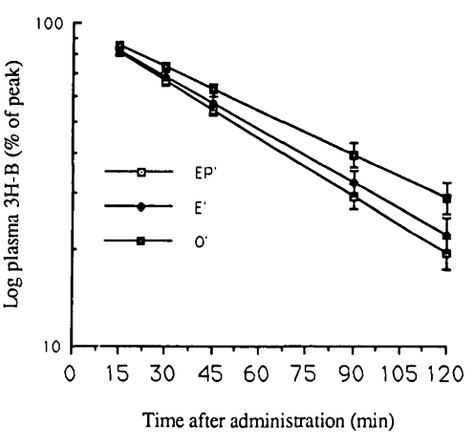


FIG. 6. Clearance of [^{3}H]B from plasma after its sc administration in OVX rats replaced with low levels of E_2 and P; O′, with high levels of E_2 and low levels of P; E′, or with high levels of E_2 and P; EP′, n = 7/group.

the distribution volumes were not affected by hormone treatment.

Discussion

Stress-induced plasma ACTH and B levels were markedly higher during the proestrous phase of the estrous cycle in the rat (Fig. 1). An E_2 treatment that mimicked the ovarian status occurring during proestrous similarly enhanced ACTH, β-END/β-LPH, and B responses to stress (see Fig. 2 and Table 2). In contrast, acute (4 h) exposure to P 20 h following E_2 administration, mimicking the late proestrous/early estrous phases, appears to inhibit the E_2-enhanced release of POMC-derived peptides during stress (Table 2, Figs. 1 and 2).

The stimulatory effect of E_2 on ACTH responses to stress seems to be transient. Animals treated with 10 μg

E_2 showed enhanced ACTH responses to stress 24 h, but not 48 h following treatment: plasma ACTH levels during stress in animals treated with E_2 48 h prior to testing were comparable to those in O′ treated animals (Fig. 3). 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 E_2 and P levels are high. The period of enhanced ACTH responses to stress might then be limited by both the waning of the E_2 effect and the inhibitory influence of elevated P 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 E_2 and P levels.

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 (22). During proestrous, the AVP/OT ratio is significantly higher, while OT messenger RNA is highest in the supraoptic nuclei during estrous (22, 23). In addition, peripheral E_2 implants acutely increase OT receptors in ventromedial hypothalamic nuclei (35). As OT and AVP secretion differ in response to various stressors (36, 37), this suggests that E_2 effects on ACTH secretion may be stress specific. Sex differences exist in circadian periodicity of CRH (38), and hypothalamic CRH content also varies during the estrous cycle (39), highest during proestrous and diestrous. It is not known, however, whether E_2 can affect CRH synthesis in the paraventricular nucleus (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 E_2 priming and subsequently depressed 4 h after P administration (40). Likewise, the percentage of PVN units responding to foot pinching are the highest during proestrous, enhanced after E_2 replacement, and depressed after P treatment in OVX rats (41). Together this demonstrates that E_2 and P specifically influence PVN activity in response to stress, in a manner consistent with our results for ACTH.

E_2 also regulates central catecholamine (CA) systems, which are a major modulator of HPA activity (reviewed in ref. 42). During proestrous, CA turnover rapidly increases prior to ovulation (43, 44). CA effects on CRH synthesis and release are dose dependent, as intracerebroventricular (ICV) administration of norepinephrine (NE) at low doses enhances CRH release (at α_1 adrenergic receptors), while at high doses release is inhibited (at β receptors) (42, 45). Interestingly, E_2 appears to up-regulate α_1-adrenergic and down regulate β-adrenergic receptors (46, 47). 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 β-(inhibitory) adrenergic receptor density, leading to an increase in ACTH synthesis.

P appears to limit enhanced ACTH responses to the early portion of proestrous. P has been shown to inhibit CRH-induced release of ACTH from cultured pituitaries (48), and to inhibit hypothalamic CRH and pituitary ACTH release *in vitro* (48, 49). *In vitro,* administration of P decreases ACTH release in the presence of low levels of B, and increases ACTH release during stress (50). P displays glucocorticoid receptor agonist activity, as it down-regulates glucocorticoid receptors in the hippocampus (51), and induces muscle glutamine synthetase activity via a glucocorticoid receptor (52). Considering the fact that the relevant P effect here occurs against a background of low (prestress) B levels, P's affinity for the glucocorticoid 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, 2, and 4). It has been proposed that P's synchronization of the hypothalamic-pituitary-gonadal (HPG) axis occurs by its suppression of E_2 receptor nuclear accumulation (reviewed in ref. 53). Thus, a similar interaction between P and E_2 receptors may be occurring within the HPA axis.

Central serotonin (5-HT) and CA systems have been implicated in mediating delayed- and fast-feedback mechanisms occurring during stress (54). Hypothalamic 5-HT content varies over the estrous cycle (55), and E_2 has been shown to cause an acute biphasic up- and down-regulation of 5-HT receptors (56). Likewise during early proestrous, E_2 increases NE and dopamine (DA) turnover rate in various hypothalamic nuclei including the suprachiasmatic nucleus and the median eminence (44, 57). Both these sites mediate CRH activity during basal and stress conditions (58). Furthermore 5-HT receptors are coexpressed in many brain regions with the glucocorticoid receptor (58). This suggests that E_2 can influence glucocorticoid feedback via its affects on 5-HT transmission. These findings provide further possible mechanisms whereby E_2 and P 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 (7, 59, 60). Wistar rats, however, have not been shown to exhibit any significant differences in basal HPA activity across the estrous cycle (38). Alternatively, it is possible that we may have missed the peak

in basal levels of ACTH and B during proestrous by sampling at 2200 h. Buckingham *et al.* (59) sampling plasma at 2 h intervals in 4-day cycling rats, showed significantly elevated plasma levels of ACTH and B during proestrous only at 1800 h, coincident with the LH surge. Endocrine differences exist between rats with 4 and 5 day cycles in terms of LH, PRL, and P release (61). Thus, lack of observed differences in basal levels of ACTH and B during the cycle in the 5-day cycling rat may very well represent another inherent difference between 4- and 5-day cycles. 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 P during the estrous cycle is to synchronize E_2 effects on the gonadotrophin cascade (31, 62, 63). Likewise, P has the same role in E_2'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-E_2 and P treated animals. Our data suggests therefore, that high physiological levels of E_2 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 glucocorticoids are known to inhibit LHRH release. Although it has been shown that ovulation is not inhibited by adrenalectomy (64), the fact that low levels of glucocorticoid agonists enhance LH and FSH release (65), suggests that the HPA axis may serve a permissive role in maintaining the metabolic needs of ovulation.

It has been repeatedly shown that stress inhibits reproductive function. Chronic exposure to stress or elevated levels of glucocorticoids disrupt the normal pattern of gonadotrophin secretion, desynchronize the estrous cycle, and decrease sexual receptivity (1–4). These effects involve activation of the HPA axis, mediated in part by CRH, as central administration of CRH inhibits LH secretion in the rodent (1). It has previously been reported that footshock applied over a 5-day period disrupts cyclicity, resulting in either a prolongation or acceleration of a single estrous stage (11). Interestingly, in the same study, a greater proportion of animals (70%) became desynchronized when the stress period was initiated during estrous or metestrous, compared to diestrous or proestrous phases (20 and 40%). Far less is known about single, acute stressors on reproductive function. In the present study, surgical stress caused by catheter implantation of intact females did not cause any apparent disruption in cyclicity as evidenced by vaginal smears. Glucocorticoid administration prior to, and up to 5 h after E_2 treatment in OVX rats, does not inhibit gonadotrophin release the following day (66). Acute surgical stress performed during proestrous has been shown to advance the onset of sexual receptivity during estrous, and is dependent on the presence of circulating steroids of adrenal origin (3, 67). Taken together, with the lack of any disruptive effect of surgical stress on cyclicity observed here, these studies reflect the abscence of any clear effects of acute stress on cyclicity.

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. Conversely, it is also possible that enhanced stress responsiveness of the HPA axis during proestrous could serve a protective mechanism ensuring subsequent reproductive success during estrous. This is consistent with the studies mentioned above concerning the effects of acute surgical stress during proestrous and the apparent phase dependency of chronic footshock stress on subsequent reproductive activity. The availability of metabolic fuels has been shown to be an important regulator of estrous cyclicity in the Syrian hamster (68). Thus, pharmacological blockade of both lipolysis and glycolysis, disrupts estrous cyclicity and sexual behavior. An enhanced glucocorticoid response to stress during proestrous which would increase carbohydrate availability, could possibly ensure an adequate supply of energy substrates required for subsequent ovulation and reproductive behavior. We are currently investigating this possibility.

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If you are interested in further information address correspondence to:

ISPNE XXIII
Department of Psychiatry
University of Wisconsin
B6/210 Clinical Science Center
600 Highland Ave.
Madison, WI 53792
FAX: Dr. Ned H. Kalin, 608-263-0265.