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Estrogen Influences the Effect of Immobilization Stress on Immunoreactive β-Endorphin Levels in the Female Rat Pituitary[1] (42653)

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Abstract. Immunoreactive β-endorphin (IR-BE) levels in the plasma, anterior pituitary (AP), the neurointermediate lobe of the pituitary (NIL), and the hypothalamus were determined in castrated female rats and castrated female rats treated with estradiol benzoate (estrogen), after exposure to acute (once for 45 min) or chronic (45 min each day for 15 consecutive days) immobilization stress. Acute and chronic stress increased plasma levels of IR-BE to the same extent in castrated female rats and castrated female rats treated with estrogen. In castrated female rats, acute stress produced an increase in the concentration of IR-BE in the AP, which was attenuated by the administration of estrogen. Although IR-BE in the NIL was not influenced by acute stress in castrated animals, exposure to acute stress resulted in an elevation in IR-BE levels in the NIL of rats given estrogen. Chronic stress did not affect the concentration of IR-BE in the AP of castrated females or castrated females treated with estrogen. Chronic stress did, however, increase the concentration of IR-BE in the NIL of castrated animals. This affect of stress on IR-BE levels in the NIL was potentiated by estrogen administration. IR-BE levels in the hypothalamus were reduced by estrogen and were not affected by acute or chronic stress, regardless of the gonadal steroid environment. As determined by column chromatography, administration of estrogen, as well as subjection to chronic stress, promoted the processing of the proopiomelanocortin precursor to form β-lipotropin rather than β-endorphin in the AP. By these methods, the only immunoreactivity detected in the NIL and the hypothalamus was β-endorphin. These data indicate that IR-BE levels in the plasma, the AP, and the NIL of female rats are affected by immobilization stress and that estrogen modulates the effects of acute immobilization stress on IR-BE levels in the AP and the NIL and the effects of chronic immobilization stress on the levels of IR-BE in the NIL.

Immobilization is a potent form of stress in the rat (1). Stress induced by immobilization has been shown to markedly alter the metabolism of dopamine (2, 3), norepinephrine (3, 4), and serotonin (2, 5) in the hypothalamus, and to promote the synthesis and release of hypothalamic corticotropin-releasing factor (CRF) (6). In the rat, dopamine (7, 8), norepinephrine (9, 10), serotonin (11, 12), and CRF (13, 14) modulate the release of the endogenous opioid peptide β-endorphin from the pituitary. It appears that the dopaminergic (15, 16) and noradrenergic (15) systems may also influence β-endorphin levels in the hypothalamus. Subjection of male rats to immobilization results in an increase of β-endorphin in the plasma (7, 11, 17, 18). It has been suggested that the increase in plasma levels of β-endorphin in response to immobilization reflects stress-induced changes in the activity of those hypothalamic nerotransmitters and neuropeptides which modulate β-endorphin levels and release (7, 11). Despite the observed elevation in β-endorphin levels in the plasma produced by immobilization stress, there have been no published reports, to our knowledge, of the effects of immobilization stress on β-endorphin levels in the anterior pituitary (AP), the neurointermediate lobe of the pituitary (NIL), or the hypothalamus.

Recent studies demonstrate that in female rats, estrogen modulates β-endorphin levels in the AP (15, 19, 20), NIL (15, 19, 20), and hypothalamus (15, 21). Estrogen also appears to regulate the ability of dopamine to control β-endorphin levels in the AP and the NIL, and the ability of the noradrenergic

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system to modulate β-endorphin levels in the AP and the hypothalamus (15). Considering that immobilization stress alters β-endorphin levels in the plasma and the activity of hypothalamic neurotransmitters and neuropeptides, and that estrogen participates in the regulation of β-endorphin levels and the modulation of β-endorphin levels by neurotransmitters, the present study proposed to test the hypothesis that acute and chronic immobilization stress may alter immunoreactive β-endorphin (IR-BE) levels in the AP, NIL, and hypothalamus and that estrogen may modify the effects of immobilization stress on levels of IR-BE.

Materials and Methods. *Animals.* Sprague–Dawley female (225–250 g) rats were obtained from Ace Animals, Inc. (Boyerstown, PA). Animals were maintained in a temperature-controlled room (22 ± 2°C) and kept on a 12:12 light:dark cycle (lights on at 0700 hr). Food (Ziegler Diet, Buckshire Farms, Landsdale, PA) and water were available *ad libitum.*

Gonadal steroids. Estradiol benzoate (estrogen) was obtained from Sigma Chemical Co. (St Louis, MO). A solution for injection was prepared by dissolving the steroid in ether and resuspending in corn oil. The ether was evaporated by continuous mixing under a stream of forced air for 48 hr. Estrogen was administered subcutaneously at a dose of 5 μg/rat/day in 100 μl of corn oil (15). Controls received 100 μl of the corn oil vehicle alone.

Castration and pretreatment with gonadal steroids. Female rats were surgically castrated under ether anesthesia and were allowed to recover for 3 weeks prior to being administered EB or vehicle for 1 week.

Induction of stress. Stress was administered by immobilizing each animal in a Plexiglas restrainer for 45 min.

Acute immobilization stress. Castrated female rats were continued on their respective treatments (gonadal steroid or vehicle; see above) for an additional 14 days. On Day 15, half of the female rats receiving gonadal steroids and half of the female rats treated with vehicle were stressed and sacrificed. The remainder of the female rats served as nonstressed controls, and were sacrificed alternately with a stressed animal receiving the same treatment (steroid or corn oil). At the time of sacrifice, female rats had been castrated for a period of 6 weeks.

Chronic stress. As in the previous set of experiments, castrated female rats were continued on their respective treatments (gonadal steroid or vehicle; see above) for 14 days. Half of the female rats treated with steroid and half of the animals treated with vehicle were also immobilized for 45 min on each of the 14 days. The remaining animals served as nonstressed controls. On Day 15, the stressed female rats were immobilized and sacrificed. The sacrifice of a stressed animal was alternated with the sacrifice of a nonstressed animal receiving the same treatment (gonadal steroid or corn oil). Similar to the experiment involving acute stress, female rats had been castrated for a period of 6 weeks at the time of sacrifice.

Collection of plasma. Animals were decapitated and blood was collected into 200 μl of 200 IU heparin (Sigma Chemical Co.) in sterile saline. The blood was centrifuged at 1000*g* for 10 min at 4°C. The resulting plasma was removed and stored at −80°C until assayed for β-endorphin.

Collection of pituitaries. The procedure for the collection of pituitaries has been described previously (15). Briefly, the AP was separated from the NIL *in situ,* using a dissecting microscope. Each lobe of the pituitary was placed in 2 ml of phosphate buffer containing 1 m*M* *N*-ethylmaleimide. Pituitary portions were then homogenized and frozen at −80°C. Pituitary protein content was measured by the technique of Lowry *et al.* (22).

Collection of hypothalami. A description of the collection of hypothalamic tissue appears in a prior publication (15). In summary, the whole brain was removed and packed in ice at the time of decapitation. After approximately 1 min, the hypothalamus was exposed and dissected. Cuts were made 3 mm anterior to the optic chiasm and posterior to the mammillary bodies. Sections were bordered laterally by the lateral hypothalamic sulci and dorsal by a cut at the top of the third ventricle. The sections included the anterior hypothalamic area, the median eminence, the infundibular stalk, and the arcuate nucleus. Tissues were homogenized in phosphate buffer containing 1 m*M* *N*-ethyl-

maleimide and stored at −80°C. Hypothalamic protein content was measured by the technique of Lowry *et al.* (22).

Radioimmunoassay (RIA) of immunoreactive β-endorphin (IR-BE). IR-BE was measured by a double antibody RIA as previously described (15). In summary, the antibody to β-endorphin was raised against human β-endorphin$_{1\text{-}31}$ (the generous gift of Dr. C. H. Li, University of California at San Francisco). On a molar basis, the antibody to human β-endorphin cross-reacted 85% with camel β-endorphin$_{1\text{-}31}$ and 100% with human β-lipotropin (generously supplied by the National Pituitary Agency). The reference preparation and hormone for iodination was camel β-endorphin$_{1\text{-}31}$ (Peninsula Laboratories, San Carlos, CA). Camel β-endorphin was iodinated using the chloramine-t method as described by Wilkes *et al.* (23). The sample volumes used produced parallel displacement to the standard curve. The minimal detectable dose of the assay was 30 pg/tube and 50% displacement of the tracer occurred at a dose of approximately 300 pg/tube. The intra- and interassay coefficients of variation were 7 and 13%, respectively.

Gel chromatography. Samples were chromatographed in a 1.6 × 70-cm glass column packed with Sephadex G-50 fine (Pharmacia Fine Pharmaceuticals, Piscataway, NJ). Aliquots of either diluted pituitary (AP = 1:100; NIL = 1:500) or hypothalamic (1:3) samples were taken from each of the 10 animals in any one group to form a pool. The column was eluted with 0.1 *N* acetic acid, 0.05% bovine serum albumin, and 0.02% sodium azide, and fractions of 1.5 ml were collected and lyophilized. The elution profiles were obtained by determining the immunoreactivity of each fraction using the β-endorphin RIA, which recognized β-endorphin (BE) and β-lipotropin. The elution profiles of the samples were compared to those obtained for either camel (ovine, bovine rat) β-endorphin or human β-lipotropin. Immunoreactivity eluting in the void volume was presumed to represent the high-molecular-weight precursor of β-endorphin and β-lipotropin, proopiomelanocortin (POMC). The recovery of β-endorphin and β-lipotropin from the column ranged from 80 to 85% (n = 5; mean = 82 ± 2).

Statistical analysis. Statistical analysis of the data was performed using a 2 × 2 ANOVA, which indicated the effects of immobilization stress, the effects of gonadal steroid treatment, and the presence of an interaction between the effects of stress and gonadal steroid treatment on IR-BE levels (15). A significance level of $P < 0.05$ was used to establish significant differences or interactions.

Results. *The effect of acute immobilization stress on IR-BE levels in the plasma, AP, NIL, and hypothalamus of castrated female rats and castrated female rats treated with estrogen.* Plasma levels of IR-BE were not different in castrated female rats and castrated female rats given estrogen, and were increased to the same level in both groups in response to acute immobilization stress. IR-BE levels in the AP of castrated female rats rose significantly after acute immobilization. Treatment with estrogen diminished the concentration of IR-BE in the AP of castrated rats, and attenuated the increase in IR-BE in the AP produced by acute stress. Acute immobilization did not affect IR-BE levels in the NIL of castrated female rats. In castrated animals given estrogen, the concentration of IR-BE in the NIL was significantly diminished as compared to nontreated castrates, and was increased significantly in response to acute stress. A significant interaction was determined between the effects of estrogen and acute stress on IR-BE levels in the AP and the NIL. The concentration of IR-BE in the hypothalamus was reduced by administration of estrogen, and was not altered in animals treated with estrogen or vehicle, subsequent to acute immobilization (Table I).

Column chromatography of the AP, NIL, and hypothalamus. Acute exposure to immobilization stress decreased the relative amount of BE in the AP. Treatment with estrogen also decreased the amount of BE in the AP. Exposure to acute stress produced a slight increase in the amount of BE relative to β-lipotropin and POMC in the AP of animals treated with estrogen (Table III). Material coeluting with BE was the only immunoreactivity observed in the NIL and hypothalamus, regardless of the treatment (data not shown).

TABLE I. THE EFFECTS OF ACUTE IMMOBILIZATION STRESS ON IR-BE LEVELS IN THE PLASMA, PITUITARY, AND HYPOTHALAMUS OF CASTRATED FEMALE RATS AND CASTRATED FEMALE RATS TREATED WITH ESTROGEN (E)[a]

	Oil	Oil + stress	E	E + stress
Plasma (pg/ml)	893 ± 70	1481 ± 158[b]	897 ± 92	1350 ± 133[c]
AP (μg/mg protein)	0.71 ± 0.10	1.10 ± 0.11[b]	0.33 ± 0.02[b]	0.58 ± 0.05[c,d]
NIL (μg/mg protein)	11.83 ± 0.81	12.52 ± 1.03	7.03 ± 1.22[b]	15.63 ± 1.26[c,d]
Hypothalamus (ng/mg protein)	2.79 ± 0.15	2.52 ± 0.10	2.02 ± 0.12[b]	2.08 ± 0.15

[a] Each value represents the mean ± SEM of 10 determinations.
[b] Differs significantly from oil-treated, nonstressed animals.
[c] Differs significantly from estrogen-treated, nonstressed animals.
[d] A significant interaction between the effects of acute stress and estrogen was determined.

The effect of chronic immobilization stress on IR-BE levels in the plasma, AP, NIL, and hypothalamus of castrated female rats and castrated female rats treated with estrogen. The concentration of IR-BE in the plasma of castrated rats was not affected by treatment with estrogen, and castrated animals and castrated animals given estrogen demonstrated a significant and equivalent elevation in plasma IR-BE in response to chronic exposure to immobilization stress. The basal concentration of IR-BE in the AP was diminished by administration of estrogen, and chronic immobilization stress did not alter the level of IR-BE in the AP of castrated rats or castrated rats treated with estrogen. The concentration of IR-BE in the NIL of castrated female rats was significantly increased by chronic exposure to stress. Administration of estrogen produced a significant reduction in the concentration of IR-BE in the NIL of castrated animals. Treatment with estrogen potentiated the increase in IR-BE demonstrated in castrated animals in response to chronic stress, indicating a significant interaction of the effects of estrogen and chronic stress on the NIL of castrated female rats. Administration of estrogen significantly lowered IR-BE levels in the hypothalamus. Chronic immobilization stress did not affect the levels of IR-BE in the hypothalamus of castrated animals or castrated animals treated with estrogen (Table II).

Column chromatography of the AP, NIL,

TABLE II. THE EFFECTS OF CHRONIC IMMOBILIZATION STRESS ON IR-BE LEVELS IN THE PLASMA, PITUITARY, AND HYPOTHALAMUS OF CASTRATED FEMALE RATS AND CASTRATED FEMALE RATS TREATED WITH ESTROGEN (E)[a]

	Oil	Oil + stress	E	E + stress
Plasma (pg/ml)	920 ± 77	1398 ± 105	807 ± 35	1257 ± 146[c]
AP (μg/mg protein)	0.64 ± 0.04	0.62 ± 0.04	0.42 ± 0.05[b]	0.39 ± 0.06
NIL (μg/mg protein)	13.38 ± 0.78	17.42 ± 0.95[b]	8.99 ± 1.23[b]	18.32 ± 1.42[c,d]
Hypothalamus (ng/mg protein)	3.36 ± 0.17	3.39 ± 0.22	2.47 ± 0.11[b]	2.69 ± 0.15

[a] Each value represents the mean ± SEM of 10 determinations.
[b] Differs significantly from oil-treated, nonstressed animals.
[c] Differs significantly from estrogen-treated, nonstressed animals.
[d] A significant interaction between the effects of chronic stress and estrogen was determined.

and hypothalamus. BE in the AP of castrated female rats was reduced by treatment with estrogen. Chronic exposure to stress decreased the amount of BE relative to β-lipotropin and POMC to the same extent in castrated female rats and castrated female rats treated with estrogen (Table III). Alterations in IR-BE in the NIL and the hypothalamus represented changes in BE, with β-lipotropin being undetectable (data not shown).

Discussion. Acute exposure to immobilization stress produced a significant elevation in the concentration of IR-BE in the AP of castrated female rats which was attenuated by treatment with estrogen. This effect is similar to our previous observation that in castrated female rats, estrogen diminishes the increase in IR-BE in the AP resulting from the chronic administration of the α-adrenergic agonist, clonidine (15). It may be suggested that the attenuation of the effect of acute stress on IR-BE levels in the AP results from a steroid-induced inhibition of IR-BE synthesis and possibly release. Steroid inhibition of IR-BE synthesis and release is demonstrated by the synthetic glucocorticoid, dexamethasone, which decreases the formation of POMC messenger RNA in the AP (24, 25) and diminishes the release of IR-BE from the AP subsequent to immobilization stress (26).

Chronic exposure to immobilization did not affect IR-BE levels in the AP of castrated female rats or castrated female rats treated with estrogen. An effect of chronic stress on the AP cannot be ruled out since IR-BE levels in the plasma were elevated and the synthesis and release of IR-BE from the AP may have been increased, with no net change in concentration.

Acute stress increased the level of IR-BE in the NIL of castrated female rats only in the presence of estrogen and chronic stress produced a greater increase in the concentration of IR-BE in the NIL of castrated animals treated with estrogen than castrated animals given vehicle alone. These differences may represent an interaction between estrogen and those neurotransmitters which regulate β-endorphin synthesis and release (7–12). In an earlier report we present data which indicate that estrogen modulates IR-BE levels in the NIL in response to chronic dopaminergic inhibition and chronic α-adrenergic stimulation (15).

Although elevated in response to stress, the effect of acute or chronic stress on plasma levels of IR-BE in castrated female rats was not influenced by gonadal steroid treatment. Mueller (27) has found that in intact male rats, testosterone did not affect the elevation in the IR-BE levels in the plasma produced by acute stress. Plasma levels of IR-BE reflect IR-BE release from the AP and the NIL (28, 29). It is possible that differences in the release of IR-BE from the AP and the NIL may result in no apparent changes in IR-BE levels

TABLE III. THE EFFECTS OF ACUTE AND CHRONIC IMMOBILIZATION STRESS ON PROOPIOMELANOCORTIN-DERIVED PEPTIDES IN THE AP OF CASTRATED FEMALE RATS AND CASTRATED FEMALE RATS TREATED WITH ESTROGEN (E)[a]

	Oil	Oil + stress	E	E + stress
Acute immobilization stress				
BE[b]	77	72	66	68
B-LPH[c]	19	24	30	29
POMC[d]	4	4	4	3
Chronic immobilization stress				
BE[b]	79	66	63	50
B-LPH[c]	18	31	32	42
POMC[d]	3	3	5	8

[a] Each value represents a percentage of the total immunoreactive β-endorphin as determined by radioimmunoassay and column chromatography.
[b] BE, β-endorphin.
[c] B-LPH, β-lipotropin.
[d] POMC, proopiomelanocortin.

in the plasma when stress is administered to castrated animals treated with gonadal steroids.

In the AP, POMC is cleaved to form BE and β-lipotropin (29). In castrated female rats, acute stress and administration of estrogen decreased the relative amount of BE in the AP. Acute stress produced a slight increase in the relative amount of BE in the AP of animals treated with estrogen. In response to chronic stress, the amount of BE relative to β-lipotropin showed a major decrease in castrated animals and decreased to a greater extent in castrated animals administered estrogen. Thus, acute stress in the absence of estrogen and chronic stress in the presence or absence of estrogen appeared to decrease the accumulation of BE in the AP, which may reflect an increase in BE secretion by the gland. In interpreting these data, however, it is necessary to consider that the chromatograms reflect BE, β-lipotropin, and POMC in an aliquot of pooled samples rather than as a composite of determinations made on each individual sample.

Acute and chronic stress did not affect IR-BE levels in the hypothalamus of castrated animals or castrated animals given estrogen. Estrogen did, however, decrease hypothalamic IR-BE levels. This finding confirms our previous report of a decrease by estrogen in hypothalamic levels of IR-BE (15) and is in agreement with the report by Wardlaw *et al.* (16) of a reduction in hypothalamic IR-BE levels in response to estrogen. The decrease in IR-BE in the hypothalamus produced by estrogen may reflect a decrease in IR-BE synthesis, since POMC messenger RNA levels are also reduced by estrogen (30).

A comparison of the effects of immobilization stress on IR-BE levels in the pituitary with the effects of other forms of stress on pituitary IR-BE levels is difficult since the duration of the exposure to stress, and in the case of studies involving chronic stress, the frequency with which the stressor was applied, often vary greatly. In addition, past studies did not involve the use of female rats and did not address the issue of gonadal steroid modulation of the effects of acute and chronic stress on pituitary and hypothalamic β-endorphin levels. Hulse and Coleman (31) have reported an interaction between gonadal steroids and β-endorphin levels in the plasma but not the AP or the NIL in response to acute stress. The differences in the data reported by Hulse and Coleman (31) and those data obtained in the present study may result from the use of different protocols for gonadal steroid treatment, the type of steroids administered (estrogen in the present study and estrogen plus progesterone in the study by Hulse and Coleman (31)), and the stress used (intermittent footshock in the previous report and immobilization in the present study). Furthermore, the ability of gonadal steroids to modulate the effects of chronic stress on BE levels was not investigated in that study.

The results of the present investigation indicate that acute and chronic immobilization stress affects IR-BE levels in the AP and the NIL. The effects of acute stress on IR-BE levels in the AP and NIL appear to be modulated by estrogen, while estrogen influences the effects of chronic stress on IR-BE levels in the NIL and not the AP. The effects of immobilization stress on IR-BE levels and the modulation of those affects by estrogen may reflect the documented ability of immobilization stress to influence hypothalamic neurotransmitter metabolism (2–5) and possibly CRF (6) and the ability of estrogen to affect the regulation by neurotransmitters of IR-BE levels (15). β-Endorphin may play an important role in the physiological response to stress (32). The data obtained in this study suggest that the endorphinergic response and possibly the physiological response to stress are modulated by gonadal steroids.

1. Pare WP, Glavin GP. Restraint stress in biomedical research: A review. Neurosci Biobehav Rev **10**:339–370, 1986.
2. Richardson JS. Brain part monoamines in the neuroendocrine mechanisms activated by immobilization stress in the rat. Int J Neurosci **23**:57–68, 1984.
3. Roth K, McIntire SL, Lorenz RG, Barchas JD. Hypothalamic catecholamine changes under acute stress occur independently of nicotinic stimulation. Neurosci Lett **28**:47–50, 1982.
4. Roth K, Mefford IM, Barchas JD. Epinephrine, norepinephrine, dopamine and serotonin: Differential effects of acute and chronic stress on regional brain amines. Brain Res **239**:417–424, 1982.
5. Sharma HS, Dey PK. Influence of long-term im-

mobilization stress on regional blood-brain barrier permeability, cerebral blood flow and 5-HT level in conscious normotensive young rats. J Neurol Sci **72**:61–76, 1985.
6. Moldow RL, Kastin AJ, Graf M, Fischman AJ. Stress mediated changes in hypothalamic corticotropin releasing factor-like immunoreactivity. Life Sci **40**:413–418, 1987.
7. Farah JM, Sapun-Malcolm D, Mueller GP. Dopaminergic inhibition of pituitary beta-endorphin-like immunoreactive secretion in the rat. Endocrinology **110**:657–59, 1984.
8. Jackson S, Lowry PJ. Secretion of pro-opiocortin peptides from isolated perfused rat pars intermedia cells. Neuroendocrinology **37**:248–257, 1983.
9. Pettibone DJ, Mueller GP. Clonidine releases immunoreactive beta-endorphin from rat pars distalis. Brain Res **221**:409–414, 1981.
10. Pettibone DJ, Mueller GP. Alpha-adrenergic stimulation by clonidine increases plasma concentrations of immunoreactive beta-endorphin in rats. Endocrinology **109**:798–802, 1981.
11. Sapun DI, Farah JM, Mueller GP. Evidence that a serotonergic mechanism stimulates the secretion of pituitary beta-endorphin-like immunoreactivity in the rat. Endocrinology **109**:421–426, 1981.
12. Lamberts SWJ, Bons EG, Uitterlinden P, Hackeng WH. Cyproheptadine and desmethylcyproheptadine inhibit the release of adrenocorticotropin and beta-lipotropin/beta-endorphin activity from the neurointermediate lobe of the rat pituitary gland. J Endocrinol **96**:395–400, 1983.
13. Vale W, Spiess J, Rivier C, Rivier J. Characterization of a 41-residue ovine hypothalamic peptide that stimulates the secretion of corticotropin and beta-endorphin. Science **213**:1396–1397, 1981.
14. Rivier C, Brownstein M, Spiess J, Rivier J, Vale W. In vivo corticotropin-releasing factor-induced secretion of adrenocorticotropin, beta-endorphin and corticosterone. Endocrinology **110**:272–278, 1982.
15. Forman LJ, Estilow S. Neurotransmitters and estrogen interact to affect beta-endorphin levels in castrated female rats. Peptides **7**:775–781, 1986.
16. Wardlaw SL, Wang PJ, Frantz AG. Regulation of beta-endorphin and ACTH in brain by estradiol. Life Sci **37**:1941–1947, 1985.
17. Mueller GP. Beta-endorphin immunoreactivity in rat plasma: Variations in response to different physical stimuli. Life Sci **29**:1669–1674, 1981.
18. DeSouza EB, Van Loon GR. Differential plasma beta-endorphin, beta-lipotropin and adrenocorticotropin responses to stress in rats. Endocrinology **116**:1577–1586, 1985.
19. Petraglia F, Penalva A, Locatelli D, Cocchi A, Panerai E, Genazzani AR, Muller EE. Effect of gonadectomy and gonadal steroid replacement on pituitary and plasma beta-endorphin levels in the rat. Endocrinology **111**:1224–1229, 1982.
20. Lim ATW, Funder JW. Estrogen lowers immunoreactive beta-endorphin in the neurointermediate lobe of the rat pituitary. Endocrinology **115**:575–580, 1984.
21. Wardlaw SL, Thoron L, Frantz AG. Effects of sex steroids on brain beta-endorphin. Brain Res **245**:327–331, 1982.
22. Lowry DH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem **193**:265–275, 1951.
23. Wilkes M, Stewart RD, Bruni JF, Quigley ME, Yen SSC, Ling N, Chretien M. A specific homologous radioimmunoassay for human beta-endorphin: direct measurement in biological fluids. J Clin Endocrinol Metab **50**:309–315, 1980.
24. Jingami H, Matsukura S, Numa S, Imura H. Effects of adrenalectomy and dexamethasone administration on the level of prepro-corticotropin-releasing factor messenger ribonucleic acid (mRNA) in the hypothalamus and adrenocorticotropin/beta-lipotropin precursor mRNA in the pituitary in rats. Endocrinology **117**:1314–1320, 1985.
25. Birnberg NC, Lissitzky JC, Hinman M, Herbert E. Glucocorticoids regulate proopiomelanocortin gene expression in vivo at the levels of transcription and secretion. Proc Natl Acad Sci USA **80**:6982–6986, 1983.
26. Mueller GP, Pettibone DJ, Farah JM, Sapun-Malcolm D. Glucocorticoid inhibition of immunoreactive beta-endorphin release from the anterior lobe of the rat pituitary: In vitro and in vivo studies. Proc Soc Exp Biol Med **179**:338–347, 1985.
27. Mueller GP. Attenuated pituitary beta-endorphin release in estrogen-treated rats. Proc Soc Exp Biol Med **165**:75–81, 1980.
28. Rossier J, French ED, Rivier C, Ling N, Guillemin R, Bloom FE. Footshock-induced stress increases beta-endorphin levels in blood but not brain. Nature (London) **270**:618–620, 1977.
29. Eipper E, Mains RE. Structure and biosynthesis of proadrenocorticotropin/endorphin and related peptides. Endocrinol Rev **1**:1–27, 1980.
30. Wilcox JN, Roberts JL. Estrogen decreases rat hypothalamic proopiomelanocortin messenger ribonucleic acid levels. Endocrinology **117**:2392–2396, 1985.
31. Hulse GK, Coleman GJ. Effect of stress on tissue and plasma levels of immunoreactive beta-endorphin in ovariectomized rats primed with oestrogen and progesterone. J Endocrinol **100**:277–280, 1984.
32. Przewlocki R. Some aspects of physiology and pharmacology of opioid peptides. Pol J Pharmacol Pharm. **36**:137–158, 1984.

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