

# ACTH, Prolactin, Corticosterone and Pituitary Cyclic AMP Responses to Repeated Stress<sup>1,2</sup>

G. JEAN KANT, EDWARD H. MOUGEY AND JAMES L. MEYERHOFF

Department of Medical Neurosciences, Walter Reed Army Institute of Research  
Washington, DC 20307-5100

Received 15 June 1988

KANT, G. J., E. H. MOUGEY AND J. L. MEYERHOFF. *ACTH, prolactin, corticosterone and pituitary cyclic AMP responses to repeated stress*. PHARMACOL BIOCHEM BEHAV 32(2) 557-561, 1989.—The present experiment was conducted to determine whether the plasma hormonal and pituitary cyclic AMP responses observed following a single exposure to an acute stressor would diminish following reexposures to the same stressor. Fifteen-min stress exposures (forced running) were separated by 45-min recovery periods. Separate groups of control and stressed animals were sacrificed before and after each of four 15-min stress periods and after each recovery period. The first exposure to 15 min of forced running raised plasma ACTH, corticosterone and pituitary cyclic AMP levels approximately 6-fold and more than tripled levels of plasma prolactin. Plasma ACTH and pituitary cyclic AMP responses to the second, third and fourth stress exposures were very similar to the responses to the first stress exposure, and levels of these substances returned to prestress levels during each 45-min recovery period. Plasma prolactin responses to the four stress sessions were somewhat variable but no significant trend among the responses was seen. Plasma prolactin levels also returned to prestress levels between stress exposures. Corticosterone levels were similar following each of the four stress sessions but levels remained elevated compared to prestress levels between stress exposures. These data suggest that pituitary responses to acute stress are rapid, that return to prestress levels is also rapid, with the exception of corticosterone, and that repeated responses of the same magnitude may be evoked when stressors are separated by short recovery periods.

ACTH    Corticosterone    Prolactin    Pituitary cyclic AMP    Stress    Rat

ACUTE stress activates a multitude of neuronal and hormonal responses (9, 12, 27, 32). The peptide corticotropin releasing factor (CRF) isolated and characterized by Vale and colleagues appears to play a key role in both autonomic and hormonal responses to acute stress (5, 10, 19, 33). CRF appears to be the primary regulator of ACTH release from the anterior pituitary gland, although other secretagogues including norepinephrine, vasopressin and angiotensin II may also affect ACTH release (6, 26, 30). CRF, released into the pituitary portal circulation, binds to specific CRF receptors located on corticotroph cells of the anterior pituitary. Binding initiates a sequence of events including stimulation of adenylate cyclase, synthesis of cyclic AMP, activation of cyclic AMP-dependent protein kinase and stimulation of release and synthesis of ACTH (2, 3, 20). Both in vitro and in vivo studies have suggested that pituitary CRF receptors may desensitize following either continuous in vitro exposure to CRF or repeated in vivo administration of exogenous CRF such that less ACTH release is seen in response to CRF

(28, 29, 34). The present experiment was conducted to determine whether desensitization to naturally-evoked pulses of CRF would be observed in vivo. Repeated exposure to an acute stressor was used to stimulate CRF release. Pituitary cyclic AMP and plasma ACTH responses were used to gauge the sensitivity of the CRF receptors. Plasma prolactin, which is not regulated by CRF, was measured as an independent estimate of the "stressfulness" of each stress challenge since it was possible that a behavioral habituation to the stressor would develop. ACTH regulates adrenal corticosterone release and this stress-responsive hormone was also measured.

## METHOD

### Animals

Male Sprague-Dawley rats (275±25 grams) were purchased from Zivic-Miller and housed for 2 weeks prior to the experiment in a light and temperature-controlled housing area. The rats were individually caged with food and water freely

<sup>1</sup>Research was conducted in compliance with the Animal Welfare Act, and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the *Guide for the Care and Use of Laboratory Animals*, NIH publication 85-23. All procedures were reviewed and approved by the WRAIR Animal Use Review Committee.

<sup>2</sup>The views of the author(s) do not purport to reflect the position of the Department of the Defense (para 4-3, AR 360-5).

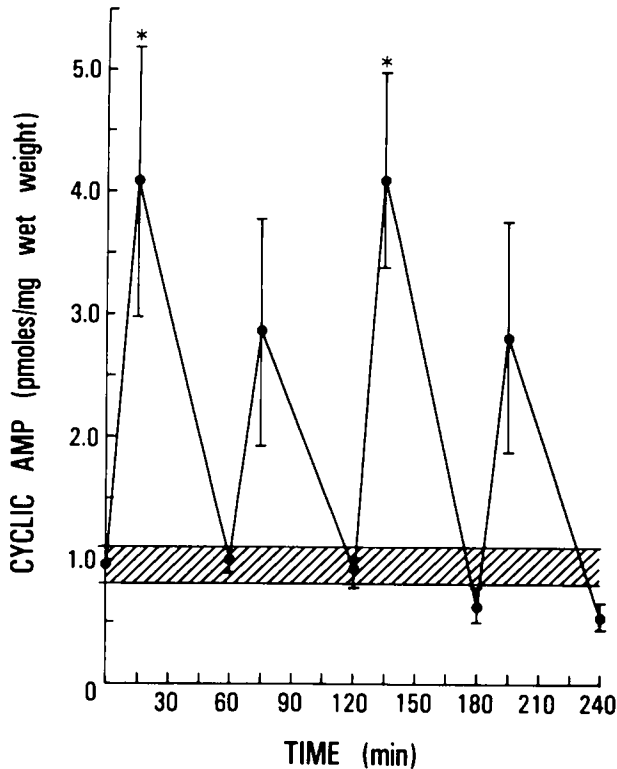


FIG. 1. Pituitary cyclic AMP response to repeated stress. A total of 80 rats were used in this experiment including 16 control and 64 experimental. Each point represents the mean  $\pm$  SEM of 16 controls or 8 stressed rats. Stressed rats were exposed to from 1 to 4 fifteen-min stress sessions  $\pm$  a 45-min recovery period prior to sacrifice. \*Significantly different from control,  $p < 0.05$ .

available. Lights were on from 0600 to 1800 hr daily. The experiment described was conducted during 4 consecutive mornings so that all animals could be sacrificed within a 2-hr period (0950 to 1150) to avoid significant circadian variations in hormonal and pituitary cyclic AMP responses to stress (13). Four control animals and two animals from each of the eight experimental groups were sacrificed each of the four days. Control (nonstressed) rats were sacrificed within 1 min of removal from their home cage to minimize nonspecific stress. Experimental animals were sacrificed either immediately after a 15-min stress session or 45 min following the end of a stress session (see below).

#### Experimental Procedures

Control rats were sacrificed by decapitation using a guillotine immediately upon removal from their home cage. Stressed rats were exposed to from one to four sessions of "forced running" (11, 14, 17, 18). Rats were placed in motor-driven metal mesh wheels (diameter 38 cm; 6 rpm, a fast walking speed) for fifteen min. Rats were either sacrificed immediately following a stress session or placed back in their home cage for a 45-min recovery period. Some rats were sacrificed after each recovery period and others were placed back into the wheel for another 15-min stress session. Thus, nine separate treatment groups were compared: control (no running), and separate groups of rats exposed to 1, 2,

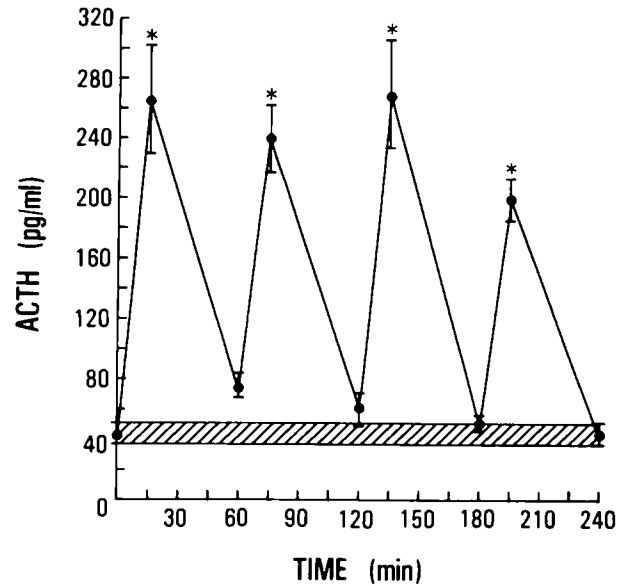


FIG. 2. Plasma ACTH response to repeated stress (see Fig. 1 for further details). \*Significantly different from control,  $p < 0.05$ .

3 or 4 running sessions with or without a 45-min recovery period prior to sacrifice. Pituitaries were removed, weighed and immediately placed into 90°C sodium acetate buffer (pH 6.2, 0.05 M) for 15 min to inactivate pituitary enzymes and prevent post-mortem changes in levels of cyclic AMP (21). In order to minimize the time required prior to heating the pituitary tissue, the anterior pituitary was not dissected free from the remaining tissue. However, we have previously shown that stress-induced increases in pituitary cyclic AMP occur in the anterior but not posterior lobe (25). Following sonication and centrifugation, supernatants were stored at  $-40^{\circ}\text{C}$  until assayed for cyclic AMP. Trunk blood was collected in heparinized beakers; Trasylol (a peptidase inhibitor) was added and plasma was stored at  $-40^{\circ}\text{C}$  until assayed for ACTH, prolactin and corticosterone.

#### Assays

Materials for the prolactin assay were provided by the National Institute of Health through the Rat Pituitary Hormone Distribution Program. Prolactin was radioiodinated as previously described (22). Within assay variation was  $< 8\%$  and between assay variation  $< 12\%$ . Corticosterone was measured by radioimmunoassay using an antibody produced in our laboratory against corticosterone-21-hemisuccinate:BSA. Somogyi reagents were used to separate free from bound ligand (24). Corticosterone [ $1,2\text{-}^3\text{H(N)}$ , specific activity 50 Ci/mmol, New England Nuclear] was the labelled ligand. Assay sensitivity was 0.6  $\mu\text{g}\%$ . The intraassay and interassay coefficients of variation were 6% and 12% respectively. ACTH was measured using a radioimmunoassay kit (Immuno Nuclear Corp). The assay was performed in 12 $\times$ 75 polypropylene tubes using an overnight incubation at 4°C. Assay sensitivity was approximately 10 pg/ml. The intraassay coefficient of variation was 2.5% at 380 pg/ml and the interassay coefficient of variation was  $< 5\%$ . Cyclic AMP was measured by radioimmunoassay using antibodies characterized in our laboratory (22).

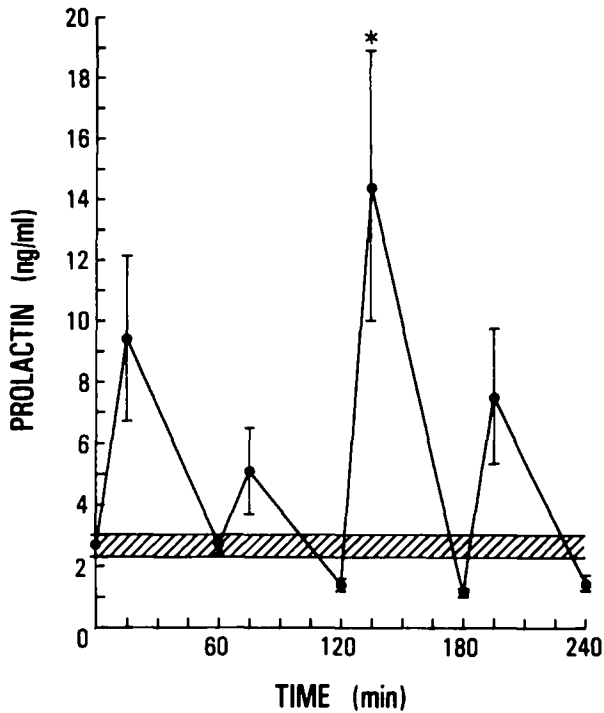


FIG. 3. Plasma prolactin response to repeated stress (see Fig. 1 for further details). \*Significantly different from control,  $p < 0.05$ .

#### Statistics

Data were analyzed by one-way analysis of variance. Following the finding of a significant F score, follow-up comparisons were made for each group vs. control. Differences were considered to be significant at  $p < 0.05$ .

#### RESULTS

As shown in Fig. 1, acute stress markedly increased levels of pituitary cyclic AMP. Forty-five min following the first 15-min stress exposure, levels of pituitary cyclic AMP had returned to prestress levels. Pituitary cyclic AMP responses to the second, third and fourth stress session were similar to the first response and levels returned to baseline between each stress session.

ACTH responses to stress mirrored the pituitary cyclic AMP responses as shown in Fig. 2. Again, no diminished ACTH response was seen following repeated stress exposures and levels of ACTH returned to prestress levels during each 45-min recovery period.

Plasma prolactin responses were somewhat variable (Fig. 3). More importantly, since prolactin response was used in this experiment to detect possible behavioral adaptation to the stress stimulus, no pattern of decreasing response was seen with increasing numbers of stress exposures. Prolactin levels also returned to prestress baselines during each recovery period.

Plasma corticosterone levels increased greatly following the first stress exposure and then failed to return to baseline during the first recovery period (Fig. 4). Levels of corticosterone immediately following exposures to subsequent stress sessions were similar to the first response, but levels of corticosterone after each 45-min recovery period decreased for each of the first three exposures.

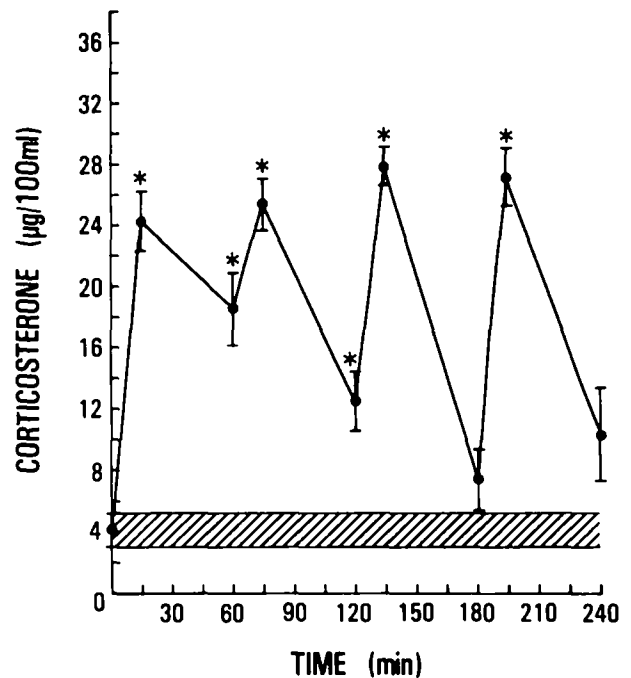


FIG. 4. Plasma corticosterone response to repeated stress (see Fig. 1 for further details). \*Significantly different from control,  $p < 0.05$ .

#### DISCUSSION

The results of this experiment confirm other studies from our laboratory (13, 15, 16, 23) which have shown that stress-evoked release of ACTH is closely linked to observable increases in levels of pituitary cyclic AMP. Both these responses are thought to be mediated via stress-induced release of CRF and its subsequent action at the pituitary corticotroph.

Regulation of the release and synthesis of ACTH from the anterior pituitary corticotroph has been shown to be a complex process. Not only do numerous secretagogues influence ACTH secretion under normal physiological status, but the relative importance of these compounds may change following disruptions of homeostasis, e.g., adrenalectomy (7). In addition, corticosterone, regulated by ACTH, feeds back upon both the brain and pituitary gland to affect the release and synthesis of ACTH (1, 4, 8).

Data from other laboratories have shown that continuous *in vitro* exposure to CRF or repeated administration of exogenous CRF or continuous exposure to stress *in vivo* results in decreased responsiveness of pituitary corticotroph receptors to CRF as measured by ACTH release (28, 29, 31, 34). In the present experiment we examined two indices of CRF receptor-stimulated function, pituitary cyclic AMP accumulation and ACTH release. We measured the response-recovery-response capabilities of this system and found that repeated full responses are seen when a recovery period is allowed between presentations of stressors. Desensitization of CRF receptors to acute stressors *in vivo* appears to be a short-lived phenomenon. Thus, the organism can respond fully to new stress challenges.

The more rapid rate of recovery of corticosterone levels toward baseline values following more exposures to the

stressor as compared to the elevated corticosterone levels at the end of the first recovery period could be interpreted as reflecting a shorter duration of ACTH release with repeated stressor presentation and thus a sign of desensitization. However, we feel that the elevated corticosterone seen at this point is more likely to be associated with mild stress (e.g., noise, movement) continuing during the recovery period which was habituated to as the experiment progressed through subsequent stress and recovery sessions. A longer duration of ACTH release would also have resulted in a larger amplitude of plasma ACTH at the 15 min measurement. Since corticosterone levels could also be affected by stress-induced changes in adrenal sensitivity to ACTH or changes in corticosterone clearance rates, we believe that the most direct indices of corticotrope response measured in this experiment are the plasma ACTH and pituitary cyclic

AMP levels. These measurements do not show any desensitization to repeated stressor presentation.

Since corticosterone levels remained elevated during the first recovery period without attenuating the ACTH response to the second stressor, the inhibitory role of glucocorticoids on ACTH release in this pattern of stressor presentation appeared to be negligible. Further studies of the regulation of this system under conditions of acute, repeated and chronic stress are underway in our laboratory.

#### ACKNOWLEDGEMENTS

The authors appreciate the expert technical support provided by Clint Wormley, Willie Gamble, Golden Driver, Clyde Kenion, SGT Gerri Fields, Terence Eggleston and Angela Brown and the secretarial support of Brenda Messer and Karen Daniels.

#### REFERENCES

- Abou-Samra, A.; Catt, K. J.; Aguilera, G. Biphasic inhibition of adrenocorticotropin release by corticosterone in cultured anterior pituitary cells. *Endocrinology* 119:972-977; 1986.
- Aguilera, G.; Harwood, J. P.; Wilson, J. X.; Morell, J.; Brown, J. H.; Catt, K. J. Mechanisms of action of corticotropin-releasing factor and other regulators of corticotropin release in rat pituitary-cells. *J. Biol. Chem.* 258:8039-8045; 1983.
- Antoni, F. A. Hypothalamic control of adrenocorticotropin secretion—Advances since the discovery of 41-residue corticotropin-releasing factor. *Endocr. Rev.* 7:351-378; 1986.
- Bilezikjian, L. M.; Vale, W. W. Glucocorticoids inhibit corticotropin-releasing factor-induced production of adenosine-3',5'-monophosphate in cultured anterior-pituitary cells. *Endocrinology* 113:657-662; 1983.
- Brown, M. R.; Fisher, L. A.; Spiess, J.; Rivier, C.; Rivier, J.; Vale, W. Corticotropin-releasing factor—Actions on the sympathetic nervous-system and metabolism. *Endocrinology* 111:928-931; 1982.
- Childs, G. V.; Burke, J. A. Use of the reverse hemolytic plaque assay to study the regulation of anterior lobe adrenocorticotropin (ACTH) secretion by ACTH-releasing factor, arginine vasopressin, angiotensin II, and glucocorticoids. *Endocrinology* 120:439-444; 1987.
- Dallman, M. F.; Makara, G. B.; Roberts, J. L.; Levin, N.; Blum, M. Corticotrope response to removal of releasing factors and corticosteroids in vivo. *Endocrinology* 117:2190-2197; 1985.
- Dallman, M. F.; Jones, M. T.; Vernikos-Danellis, J.; Ganong, W. F. Corticosteroid feedback control of ACTH secretion: Rapid effects of bilateral adrenalectomy on plasma ACTH in the rat. *Endocrinology* 91:961-968; 1972.
- Du Ruisseau, P.; Tache, Y.; Brazeau, P.; Collu, R. Pattern of adenohipophyseal hormone changes induced by various stressors in female and male rats. *Neuroendocrinology* 27:257-271; 1978.
- Fisher, L. A.; Rivier, J.; Rivier, C.; Spiess, J.; Vale, W. Corticotropin-releasing factor (CRF)—Central effects on mean arterial-pressure and heart-rate in rats. *Endocrinology* 110:2222-2224; 1982.
- Kant, G. J.; Bunnell, B. N.; Mougey, E. H.; Pennington, L. L.; Meyerhoff, J. L. Effects of repeated stress on pituitary cyclic AMP and plasma prolactin, corticosterone and growth hormone in male rats. *Pharmacol. Biochem. Behav.* 18:967-971; 1983.
- Kant, G. J.; Mougey, E. H.; Pennington, L. L.; Meyerhoff, J. L. Graded footshock stress elevates pituitary cyclic AMP and plasma beta-endorphin, beta-LPH, corticosterone and prolactin. *Life Sci.* 33:2657-2663; 1983.
- Kant, G. J.; Mougey, E. H.; Meyerhoff, J. L. Diurnal variation in neuroendocrine response to stress in rats: Plasma ACTH, beta-endorphin, beta-LPH, corticosterone, prolactin and pituitary cyclic AMP responses. *Neuroendocrinology* 43:383-390; 1986.
- Kant, G. J.; Meyerhoff, J. L.; Bunnell, B. N.; Lenox, R. H. Cyclic AMP and cyclic GMP response to stress in brain and pituitary: Stress elevates pituitary cyclic AMP. *Pharmacol. Biochem. Behav.* 17:1067-1072; 1982.
- Kant, G. J.; Oleshansky, M. A.; Walczak, D. D.; Mougey, E. H.; Meyerhoff, J. L. Comparison of the effects of CRF and stress on levels of pituitary cyclic AMP and plasma ACTH in vivo. *Psychiatry Res.* 16(Suppl. 4):64; 1986.
- Kant, G. J.; Oleshansky, M. A.; Walczak, D. D.; Mougey, E. H.; Meyerhoff, J. L. Comparison of the effects of CRF and stress on levels of pituitary cyclic AMP and plasma ACTH in vivo. *Peptides* 7:1153-1158; 1986.
- Kant, G. J.; Lenox, R. H.; Bunnell, B. N.; Mougey, E. H.; Pennington, L. L.; Meyerhoff, J. L. Comparison of stress response in male and female rats: Pituitary cyclic AMP and plasma prolactin, growth hormone and corticosterone. *Psychoneuroendocrinology* 8:421-428; 1983.
- Kant, G. J.; Eggleston, T.; Landman-Roberts, L.; Kenion, C. C.; Driver, G. C.; Meyerhoff, J. L. Habituation to repeated stress is stressor specific. *Pharmacol. Biochem. Behav.* 22:631-634; 1985.
- Knepel, W.; Homolka, L.; Vlasovsk, M.; Nutto, D. Stimulation of adrenocorticotropin beta-endorphin release by synthetic ovine corticotropin-releasing factor in vitro—Enhancement by various vasopressin analogs. *Neuroendocrinology* 38:344-350; 1984.
- Labrie, F.; Veilleux, R.; Lefevre, G.; Coy, D. H.; Sueiras-Diaz, J.; Schally, A. V. Corticotropin-releasing factor stimulates accumulation of adenosine 3',5'-monophosphate in rat pituitary corticotrophs. *Science* 216:1007-1008; 1982.
- Lenox, R. H.; Kant, G. J.; Meyerhoff, J. L. In: Lajtha, A., ed. *Handbook of neurochemistry*. vol. 2. New York: Plenum Press; 1982:77-102.
- Lenox, R. H.; Kant, G. J.; Sessions, G. R.; Pennington, L. L.; Mougey, E. H.; Meyerhoff, J. L. Specific hormonal and neurochemical responses to different stressors. *Neuroendocrinology* 30:300-308; 1980.
- Meyerhoff, J. L.; Mougey, E. H.; Kant, G. J. Paraventricular lesions abolish the stress-induced rise in pituitary cyclic adenosine monophosphate and attenuate the increases in plasma levels of proopiomelanocortin-derived peptides and prolactin. *Neuroendocrinology* 46:222-230; 1987.

24. Mougey, E. H. A radioimmunoassay for tetrahydrocortisol. *Anal. Biochem.* 91:566-582; 1978.
25. Mougey, E. H.; Meyerhoff, J. L.; Pennington, L. L.; Gamble, W. L.; Driver, G. C.; Kant, G. J. Stress-induced increases in pituitary cyclic AMP occur in the anterior but not the posterior lobe. *Soc. Neurosci. Abstr.* 12:1059; 1986.
26. Murakami, K.; Hashimoto, K.; Ota, Z. Interaction of synthetic ovine corticotropin releasing-factor and arginine vasopressin on in vitro ACTH release by the anterior pituitary of rats. *Neuroendocrinology* 39:49-53; 1984.
27. Natelson, B. H.; Tapp, W. N.; Adamus, J. E.; Mittler, J. C.; Levin, B. E. Humoral indices of stress in rats. *Physiol. Behav.* 26:1049-1054; 1981.
28. Reisine, T.; Hoffman, A. Desensitization of corticotropin-releasing factor receptors. *Biochem. Biophys. Res. Commun.* 111:919-925; 1983.
29. Rivier, C.; Vale, W. Influence of the frequency of ovine corticotropin-releasing-factor administration on adrenocorticotropin and corticosterone secretion in rat. *Endocrinology* 113:1422-1426; 1983.
30. Rivier, C.; Vale, W. Modulation of stress-induced ACTH release by corticotropin-releasing factor, catecholamines and vasopressin. *Nature* 305:325-327; 1983.
31. Rivier, C.; Vale, W. Diminished responsiveness of the hypothalamic-pituitary-adrenal axis of the rat during exposure to prolonged stress: a pituitary-mediated mechanism. *Endocrinology* 121:1320-1328; 1987.
32. Seggie, J. A.; Brown, G. M. Stress response patterns of plasma corticosterone, prolactin, and growth-hormone in rat, following handling or exposure to novel environment. *Can. J. Physiol. Pharmacol.* 53:629-637; 1975.
33. Vale, W.; Spiess, J.; Rivier, C.; Rivier, J. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. *Science* 213:1394-1397; 1981.
34. Vale, W.; Vaughan, J.; Smith, M.; Yamamoto, G.; Rivier, J.; Rivier, C. Effects of synthetic ovine corticotropin-releasing factor, glucocorticoids, catecholamines, neurohypophyseal peptides, and other substances on cultured corticotropic cells. *Endocrinology* 113:1121-1131; 1983.