for studies of the acquisition of sensitivity to LH during development. These workers observed the concomitant appearance of LH-stimulated cyclic AMP and testosterone production on day 15 of gestation, and noted that exposure of 14-day testes to LH did not decrease their subsequent responsiveness to gonadotropic stimulation when differentiation had taken place. In contrast, Paz *et al.* (*13*) observed that the testosterone response of 19-day rat testes decreased during the second and third days of culture with 2 IU of hCG, suggesting the presence of a desensitization process as observed in the adult testis. However, the results of the present study clearly demonstrate that retention of LH receptors and enhanced steroidogenic responses occur in the gonadotropin-stimulated fetal Leydig cell, similar to the responses of the neonatal testis in vivo (*6*). The absence of steroidogenic defects in the fetal testis could be due to the relative lack of estrogen receptors in the immature Leydig cell (*14*), since this lesion appears to be caused through nuclear actions of testicular estrogen following hormonal stimulation (*3–5*) and may only become apparent during postnatal development. Such a feature of the developing gonad could facilitate maintenance of high androgen levels during stimulation by CG, which is detectable in the placenta and maternal circulation of the rat from day 7 of gestation (*15*), prior to sexual differentiation. If the placental hormone is constantly available to the fetal testis, in contrast to the pulsatile adult mode of LH secretion, the ability of the fetal Leydig cell to respond to sustained concentrations of gonadotropin without being desensitized would contribute to the maintenance of elevated androgen production during early development.

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References and Notes

1. D. Price and R. Pannabecker, *Arch. Anat. Microsc. Morphol. Exp.* **98**, 223 (Suppl.) (1959); A. Jost, *Recent Prog. Horm. Res.* **8**, 379 (1953).
2. A. J. W. Hsueh, M. L. Dufau, K. J. Catt, *Biochem. Biophys. Res. Commun.* **72**, 1145 (1976); T. Tsuruhara, M. L. Dufau, S. B. Cigorraga, K. J. Catt, *J. Biol. Chem.* **252**, 9002 (1977).
3. S. B. Cigorraga, M. L. Dufau, K. J. Catt, *J. Biol. Chem.* **253**, 4297 (1978); M. L. Dufau, S. Cigorraga, A. J. Baukal, S. Sorrel, J. M. Bator, K. J. Catt, *Endocrinology* **105**, 1314 (1979); K. Nozu, S. Matsuura, K. J. Catt, M. L. Dufau, *J. Biol. Chem.* **256**, 10012 (1981); K. Nozu, M. L. Dufau, K. J. Catt, *ibid.*, p. 1915.
4. E. H. Charreau, J. C. Calvo, K. Nozu, O. Pignataro, K. J. Catt, M. L. Dufau, *J. Biol. Chem.* **256**, 12719 (1981).
5. K. Nozu, A. Dehejia, L. Zawistowich, K. J. Catt, M. L. Dufau, *ibid.*, p. 12875.
6. I. T. Huhtaniemi, M. Katikineni, K. J. Catt, *Endocrinology* **109**, 588 (1981).
7. E. C. Roosen-Runge and D. Anderson, *Acta Anat.* **37**, 125 (1959).
8. Highly purified hCG (CR119, 12,000 IU/mg) was obtained from the Center for Population Research, NICHD, Bethesda, Md. Ovine LH (S18, 1.03 NIH S1 units per milligram) and ovine FSH (S14, 7 NIH S1 units per milligram) were obtained from the National Pituitary Agency through the Hormone Distribution Program of the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases.
9. The hCG dose of 100 ng/ml was shown to cause maximum stimulation of testosterone production in fetal testes.
10. M. Knecht, A. Amsterdam, K. J. Catt, *J. Biol. Chem.* **256**, 10628 (1981).
11. D. W. Warren and E. Loumaye, Endocrine Society, 63rd Annual Meeting, Cincinnati, Ohio, 1981, Abstract 60.
12. R. Picon and M.-N. Gangerau, *Mol. Cell. Endocrinol.* **18**, 137 (1980).
13. G. Paz, J. Thliveris, J. Winter, F. Reyes, C. Faiman, *Biol. Reprod.* **23**, 1087 (1980).
14. I. T. Huhtaniemi, K. Nozu, D. W. Warren, M. L. Dufau, K. J. Catt, *Endocrinology*, in press.
15. F. Haour, G. Tell, P. Sanchez, *C. R. Acad. Sci. Ser. D* **282**, 1183 (1976); M. S. Blank, M. L. Dufau, H. G. Friesen, *Life Sci.* **25**, 1023 (1979); M. S. Blank, E. Loumaye, K. J. Catt, M. L. Dufau, *Factors Regulating Ovarian Function. Proceedings of the Fourth Ovarian Workshop*, G. Greenwald, Ed. (Raven, New York, in press).
16. We thank G. Knox and S. Luna for excellent technical assistance. D.W.W. was the recipient of a National Research Service Fellowship Award No. 1F33-HD06192.

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Inhibition of Adrenocorticotropic Hormone Secretion in the Rat by Immunoneutralization of Corticotropin-Releasing Factor

Abstract. *Intravenous administration of rabbit antiserum to ovine corticotropin-releasing factor (CRF) markedly reduced the CRF-induced rise of plasma adrenocorticotropic hormone (ACTH) in intact nonstressed adult male rats while blocking more than 75 percent of the ACTH release observed in rats exposed to ether stress. Furthermore, antiserum to CRF significantly lowered ACTH levels in adrenalectomized animals. These results suggest that endogenous CRF plays a physiological role in regulating ACTH secretion.*

The key role played by the hypothalamus in the regulation of adrenocorticotropic hormone (ACTH) secretion is well established [reviews in (*1*)]. We reported the isolation, characterization, and synthesis of a 41 amino acid peptide from ovine hypothalamic extracts with high potency and intrinsic activity for stimulating the secretion of corticotropin and β-endorphin-like immunoreactivity both in vitro and in vivo (*2–4*). Corticotropin-releasing factor (CRF)–like immunoactivity has been observed in the hypothalamus of the rat, dog, monkey, and human being either by radioimmunoassay or by immunocytochemistry (*5*); it has also been observed in pituitary stalk blood of the rat (*6*). We therefore used antiserum to CRF to investigate the role played by endogenous peptides immunologically related to this CRF in regulating ACTH secretion under various circumstances (*7*).

Adult Sprague-Dawley male rats (230 to 250 g) were given free access to Purina Chow and water. When appropriate, adrenalectomy was performed (by the lumbar approach) in ether-anesthesized animals 3 weeks before the experiment. All animals were equipped with jugular indwelling venous cannulas 36 hours before the experiment and were placed in individual cages in a quiet environment. The cannulas were used both for administration of the treatments and for serial blood sampling.

All three experiments were carried out in the afternoon. In experiment 1A, groups of six intact rats first received 1 ml of normal rabbit serum (NRS) or 1 ml of antiserum to CRF and then, 15 minutes later, saline, synthetic ovine CRF (*8*), or a 3-minute exposure to ether. In experiment 1B, intact rats first received Na_2SO_4-precipitated NRS or antiserum to CRF and then, 1 minute later, saline, CRF, or a 3-minute exposure to ether. In experiment 2, 1.5 ml of NRS or of antiserum to CRF was administered to nonstressed adrenalectomized animals. Since only 0.5 ml of blood was removed during each of the four subsequent samplings, this volume was not replaced.

Figure 1A illustrates the effect of prior treatment with NRS or antiserum to CRF on ACTH secretion (*9*), which was measured 10 minutes after the animal was given saline or 0.45 nmole of CRF or was subjected to ether stress (experiment 1A). In contrast to treatment with NRS, the administration of antiserum to CRF prevented most of the CRF-induced rise in ACTH levels induced by administration of CRF [mean ± standard error (S.E.) of 1305 ± 169 pg/ml versus 182 ± 51 pg/ml; $P \leq .01$] or by ether stress (1729 ± 184 pg/ml versus 363 ± 102 pg/ml; $P \leq .01$). In experiment 1B (Fig. 1B), Na_2SO_4-precipitated NRS or antiserum to CRF was administered in amounts (on a protein basis) equivalent to 1.5 ml of serum, and plas-

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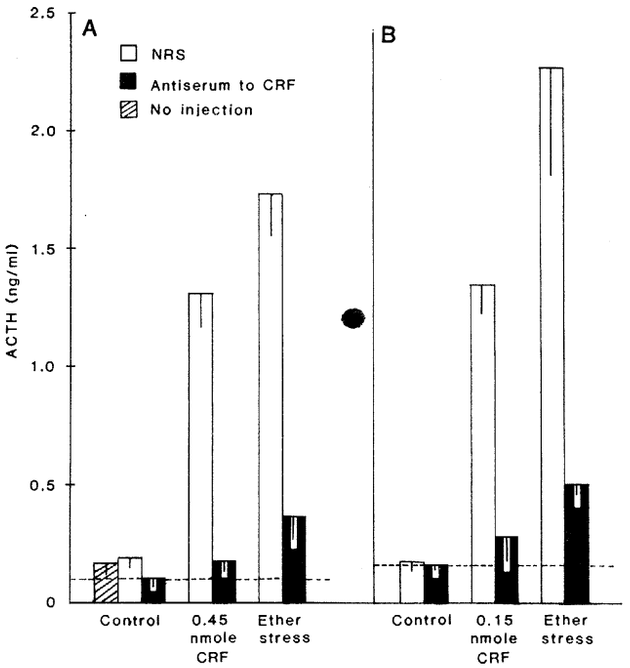

Fig. 1. (A) Effects of NRS or antiserum to CRF (injected into intact rats 15 minutes before the stimuli) on plasma ACTH in samples taken 10 minutes after exposure to saline, synthetic ovine CRF, or ether stress. (B) Effects of Na_2SO_4-precipitated NRS or antiserum to CRF (injected into intact rats 1 minute before the stimuli) on plasma ACTH in samples taken 5 minutes after exposure to saline, synthetic ovine CRF, or ether stress. (Open bars) rats injected with Na_2SO_4-precipitated NRS; (closed bars) rats injected with Na_2SO_4-precipitated antiserum to CRF. Each bar represents the mean ± S.E. of six animals. Data were evaluated by analysis of variance. The dashed line indicates the minimal detectable ACTH concentration in the radioimmunoassay.

ma ACTH was measured 5 minutes later. Again, the administration of antiserum to CRF blocked the rise in plasma ACTH resulting either from CRF administration (1392 ± 191 pg/ml versus 292 ± 144 pg/ml; $P \leq .01$) or ether stress (2262 ± 439 pg/ml versus 501 ± 45 pg/ml; $P \leq .01$). In both experiments, rats injected with antiserum to CRF plus CRF had plasma ACTH concentrations that were statistically comparable to those of control animals, whereas rats stressed after administration of the antiserum maintained a slightly elevated ACTH output. In nonstressed adrenalectomized rats (experiment 2 in Fig. 2), antiserum to CRF produced a rapid drop in ACTH secretion, which was statistically significant for 15 to 240 minutes after the injection. At 15 minutes, the value for the NRS control was 3299 ± 519 pg/ml, and for the group receiving antiserum was 1332 ± 166 pg/ml ($P \leq .01$); at 240 minutes, the value for the NRS control was 1787 ± 776 pg/ml, and for the group receiving antiserum was 173 ± 34 pg/ml ($P \leq .01$).

These results indicate that administration of antiserum to CRF reduces the ACTH secretion induced by exogenous synthetic ovine CRF to levels that are statistically comparable to control values, blocks most of the stress-induced ACTH rise in intact rats, and inhibits the increased ACTH secretion observed after removal of corticosteroid feedback. Such data are consistent with a physiological role of endogenous CRF-like molecules in the activation of the pituitary-adrenal axis due to stress as well as in the maintenance of an elevated ACTH production in adrenalectomized rats. The small residual ACTH secretion observed in intact animals treated with antiserum to CRF and then exposed to stress might be due to the inability of our antiserum (produced against synthetic ovine CRF) to rapidly neutralize all of the available endogenous rat CRF; alternatively, a factor such as vasopressin, epinephrine, or norepinephrine, distinct from CRF, could contribute to the activation of pituitary corticotropic cells under stressful circumstances.

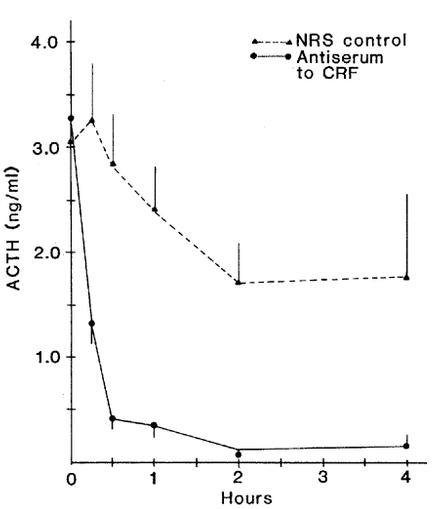

Fig. 2. Effects of NRS or antiserum to CRF on plasma ACTH in adrenalectomized rats. Each point represents the mean ± S.E. of six animals.

Several substances present in hypothalamic extracts or peripheral blood have been suggested as putative ACTH-releasing factors, including CRF, vasopressin, and catecholamines (*2–4, 10*). For various reasons, including their low intrinsic activity in vitro, neither vasopressin, nor epinephrine or norepinephrine, are considered likely to be the major physiological regulators of ACTH secretion (*2–4, 11*). However, several studies have implicated vasopressin as a possible potentiator of the actions of CRF on the pituitary gland (*1, 11*), and we have observed (*12*) that vasopressin and epinephrine or norepinephrine potentiate the action of synthetic CRF in vitro. Endogenous vasopressin or the catecholamines may be responsible for the greater amounts of ACTH observed after ether stress than after CRF injection, as well as for the residual release of ACTH in stressed rats treated with antiserum to CRF.

The observation that administration of antiserum to CRF can lower plasma ACTH levels in either adrenalectomized or intact stressed rats emphasizes the physiological significance of a structurally related endogenous peptide in regulating the secretory activity of corticotropic cells.

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References and Notes

1. G. W. Harris, *Physiol. Rev.* **28**, 139 (1948); F. E. Yates and J. W. Maran, in *Handbook of Physiology*, E. Knobil and W. H. Sawyer, Eds. (American Physiological Society, Bethesda, Md., 1974), vol. 4, sect. 7; W. Vale, C. Rivier, M. Brown, *Annu. Rev. Physiol.* **29**, 473 (1977).
2. W. Vale and C. Rivier, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **36**, 2094 (1977); J. Spiess, J. Rivier, C. Rivier, W. Vale, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6517 (1981); C. M. Turkelson *et al., Peptides* **2**, 425 (1981).
3. W. Vale, J. Spiess, C. Rivier, J. Rivier, *Science* **213**, 1394 (1981).
4. C. Rivier, M. Brownstein, J. Spiess, J. Rivier, W. Vale, *Endocrinology* **110**, 272 (1982).
5. F. Bloom, A. Battenberg, J. Rivier, W. Vale, *Regul. Peptides* **4**, 43 (1982); W. Vale *et al.*, in preparation.
6. D. Gibbs and W. Vale, *Endocrinology*, in press.
7. Antiserum to synthetic ovine CRF was raised in rabbits injected intradermally every 2 weeks with CRF coupled by glutaraldehyde to human α-globulins. α-Globulin antibodies were precipitated by treatment of antiserum with 1 mg of human α-globulin per milliliter for 24 hours, followed by centrifugation. In experiment 2, α-globulin fractions of both antiserum to CRF and NRS were obtained by precipitation with Na_2SO_4 (40 percent) and subsequent dialysis against 0.9 percent NaCl. Radioimmunoassays with this antiserum (C-30) are highly specific for ovine CRF, exhibiting less than 0.01 percent cross reactivity with any other tested peptide, including vasopressin, angiotensin, and sauva-

gine. Studies with highly purified rat CRF in a bioassay and several radioimmunoassays indicate that CRF antiserum C-30 may have less than 30 times the affinity for rat CRF than it has for ovine CRF.
8. CRF was synthesized (*3*) and dissolved in a mixture of 0.1 percent bovine serum albumin and 0.1 percent ascorbic acid and saline.
9. ACTH was measured by radioimmunoassay (*4*), with sheep antiserum to ACTH. The minimum detectable amount of ACTH was 50 pg/ml.
10. B. Nichols and R. Guillemin, *Endocrinology* **64**, 914 (1959); B. L. Nichols, *Yale J. Biol. Med.* **33**, 415 (1961); A. V. Schally, A. Arimura, C. Y. Bowers, A. J. Kastin, S. Sawano, T. W. Redding, *Recent Prog. Horm. Res.* **24**, 497 (1968); J. C. Buckingham, *Pharmacol. Rev.* **31**, 253 (1980); G. Gillies, T. B. Van Wimersma Greidanus, P. J. Lowry, *Endocrinology* **103**, 528 (1978).
11. J. C. Buckingham and J. H. Leach, *J. Physiol. (London)* **296**, 87P (1979); G. Gillies and P. Lowry, *Nature (London)* **278**, 463 (1979).
12. W. Vale, C. Rivier, J. Spiess, M. Brown, J. Rivier, in *Brain Peptides*, D. Krieger *et al.*, Eds. (Wiley, New York, in press).
13. Supported in part by NIH grants AM26741, AM20917, AM18811, AA03504, and HD13527; by the Rockefeller Foundation; and by the March of Dimes Birth Defects Foundation. Research conducted in part by the Clayton Foundation for Research, California division. C.R. and W.V. are Clayton Foundation investigators. We thank M. Tam, N. Keating, S. Quijada, P. Hernandez, R. Kaiser, R. Galyean, M. Newbury, R. McClintock, G. Yamamoto, J. Vaughan, and C. Douglas for technical assistance and L. Wheatley and S. McCall for manuscript preparation. We are also indebted to D. Orth for providing the antiserum to ACTH.

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Direct Measurement of Pulmonary Capillary Transit Times

Abstract. *Direct measurements of capillary transit times in dog lungs were made by using in vivo television microscopy. Mean transit times were unexpectedly long. As pulmonary artery pressures were raised, transit times decreased, suggesting that the normally existing hydrostatic pressure gradient in the lung causes a vertical distribution of transit times. The more rapid transit times approached the minimum time required for complete oxygenation.*

The time that blood spends in the pulmonary capillaries is a major determinant of the effectiveness of the gas exchange in the lung. Complete oxygenation occurs in a remarkably short time. Red cells spend about 0.75 second in the pulmonary capillaries in resting subjects and only 0.3 second during exercise (*1, 2*). These values, however, are based on indirect measures of mean capillary transit times for the lung as a whole. They provide no information about the range of transit times in individual capillary beds, in different hydrostatic zones of the lung, or during altered hemodynamic states. The most rapid transit times are especially important, for if some red cells pass through the gas-exchange vessels too quickly, arterial hypoxemia can result. Very rapid red cell transit may be involved in the exercise-induced arterial hypoxemia found in patients with restricted pulmonary vascular beds, in normal individuals (*3, 4*), and even some world-class runners (*5*). To confirm the indirect estimates of mean pulmonary capillary transit times and to determine the range of transit times, we made direct measurements of the passage of blood across single pulmonary capillary networks by using television microscopy in vivo.

Eight pentobarbital-anesthetized dogs (mean weight, 24 kg) were prepared for measurement of systemic and pulmonary arterial pressures, blood gases, and cardiac output (*6*). These measurements were in the normal range. A transparent window was then implanted in the ninth left intercostal space to permit alveolar capillaries on the surface of the left lower lobe to be studied (*7, 8*). The animals were placed in the right lateral decubitus position so that the microscope looked down through the window at the uppermost surface of the lung. To determine capillary transit time a fluorescent dye, fluorescein isothiocyanate in dextran (*9*), was injected through a catheter placed in the left lower lobe artery under fluoroscopic guidance (*10*). Passage of the dye through the microcirculation was observed with a Leitz Ultropak microscope coupled to a COHU 4400 television camera and was recorded on videotape with a Panasonic PV-1210 video (VHS) recorder. To measure capillary transit time, it was necessary to determine when an average molecule in the dye bolus entered and exited a single capillary bed. As the videotape was replayed, the dye passage through the arteriole (diameter, < 20 μm) and a small venule (< 15 μm) serving a common capillary network was electronically sampled. The signals were passed through a sample-and-hold circuit at 60 Hz and then recorded oscillographically to produce dye dilution curves (Fig. 1). A signal indicating the time of dye injection was placed on the audio channel of the videotape and later transcribed with the dye dilution curves. The time at which the average molecule passed the sample window was determined by calculating the first moment (time-weighted mean) of each curve. Mean capillary transit time was obtained by subtracting the first moments of the arteriolar and venular curves with reference to the common injection time signal.

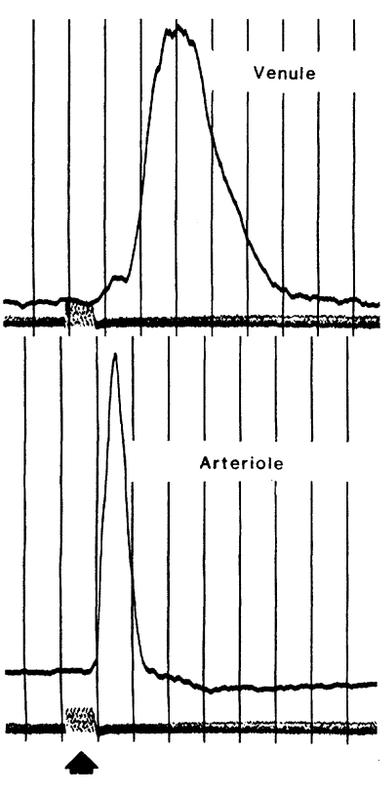

Fig. 1. Photometric records of passage of fluorescein isothiocyanate through a venule and an arteriole. Time lines represent individual seconds. The venular curves were consistently wider than the arteriolar curves, indicating that some of the dye was delayed in its passage through the capillary bed—that is, that there was a distribution of transit times in the capillary bed.

The average of the mean capillary transit times for eight dogs under control conditions was 12.7 ± 3.2 seconds (mean $\pm$ standard error) at an average pulmonary artery pressure of 7.7 ± 1.0 torr (*11*). This transit time was unexpectedly long—more than an order of magnitude longer than the 0.75 second for whole lung capillary transit found by indirect techniques. In fact, the mean capillary transit time we measured is even longer than the mean transit time across the whole lung; mean transit time from the main pulmonary artery to the left atrium was shown by Maseri *et al.* (*12*) to be 6.4 seconds and by Knopp and Bassingthwaighte (*13*) to be 6.8 seconds.

The differences between our measurements and those made with other techniques may be explained by the observation that plasma travels across the lung more slowly than red cells (*14*). Fluorescein isothiocyanate in dextran labels

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