tion. Arachidonic acid (1 or 10 μ*M*) and A23187 (0.1 μ*M*) were each added to three wells. The conditioned medium was then concentrated and measured for iSRS activity (*11*). These experiments were repeated twice.
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Synthetic Competitive Antagonists of Corticotropin-Releasing Factor: Effect on ACTH Secretion in the Rat

Abstract. *Polypeptide analogs of the known members of the corticotropin-releasing factor (CRF) family were synthesized and tested in vitro and in vivo for enhanced potency or competitive antagonism. Predictive methods and physicochemical measurements had suggested an internal secondary α-helical conformation spanning about 25 residues for at least three members of the CRF family. Maximization of α-helix–forming potential by amino acid substitutions from the native known sequences (rat/human and ovine CRF, sauvagine, and carp and sucker urotensin 1) led to the synthesis of an analog that was found to be more than twice as potent as either of the parent peptides in vitro. In contrast, certain amino-terminally shortened fragments, such as α-helical CRF or ovine CRF residues 8 to 41, 9 to 41, and 10 to 41, were found to be competitive inhibitors in vitro. Selected antagonists were examined and also found to be active in vivo.*

Corticotropin-releasing factor (CRF), a 41-residue peptide first characterized in ovine hypothalamic extracts (oCRF) (*1*), is the principal neuroregulator of the secretion of adenocorticotropic hormone (ACTH), β-endorphin, and other pro-opiomelanocortin products of the anterior pituitary gland (*2, 3*). Rat hypothalamic CRF (rCRF) has been isolated and sequenced (*4*), and an identical structure has been proposed for human CRF on the basis of the DNA sequence of the human CRF genome (*5*). Mammalian CRF's have approximately 50 percent homology with the frog skin peptide sauvagine (*6*) and the fish urophysial peptide urotensin 1 (U_1) (*7*); all of these peptides are equipotent stimulators of ACTH secretion in vivo and in vitro (*3*). The broad distribution in the central nervous system (*8*) of CRF and several demonstrated autonomic (*9*) and behavioral (*10*) actions of CRF suggest that this peptide may play important roles within the brain, especially during stress.

Peptide analogs are generally designed to fulfill certain needs that are either not satisfied or only partially satisfied by the parent compound. Analogs with higher affinity for their receptor and higher resistance to biodegradation may be more potent and longer acting. Some other substitutions may result in better chemical stability (for example, substitution of a methionine by norleucine or norvaline). Similarly, competitive antagonists of several regulatory peptides have been developed and shown to be useful for studying the physiologic roles of the corresponding endogenous peptides (*11, 12*) or for therapeutic applications (*13*).

Although antibodies to CRF proved useful in early studies of the role of endogenous CRF (*2*), they are of limited value because of their size, species specificity, antigenicity, and poor distribution in the brain (even when administered in the cerebral ventricles). Competitive antagonists of CRF were therefore developed to facilitate studies of the physiologic and pathophysiologic significance of endogenous CRF in experimental animals and, possibly, in human beings.

We have used pituitary cells in vitro (*14*) to assay the potency of our synthetic analogs relative to synthetic oCRF and to discover partial agonists and antagonists (*11*). In vivo experiments were performed as described (*2, 15*) (see Figs. 1 and 2). Peptides were synthesized by the solid phase method (*16*); purification of the crude synthetic peptides generated after treatment with HF and cleavage from the resin was achieved by prepara-

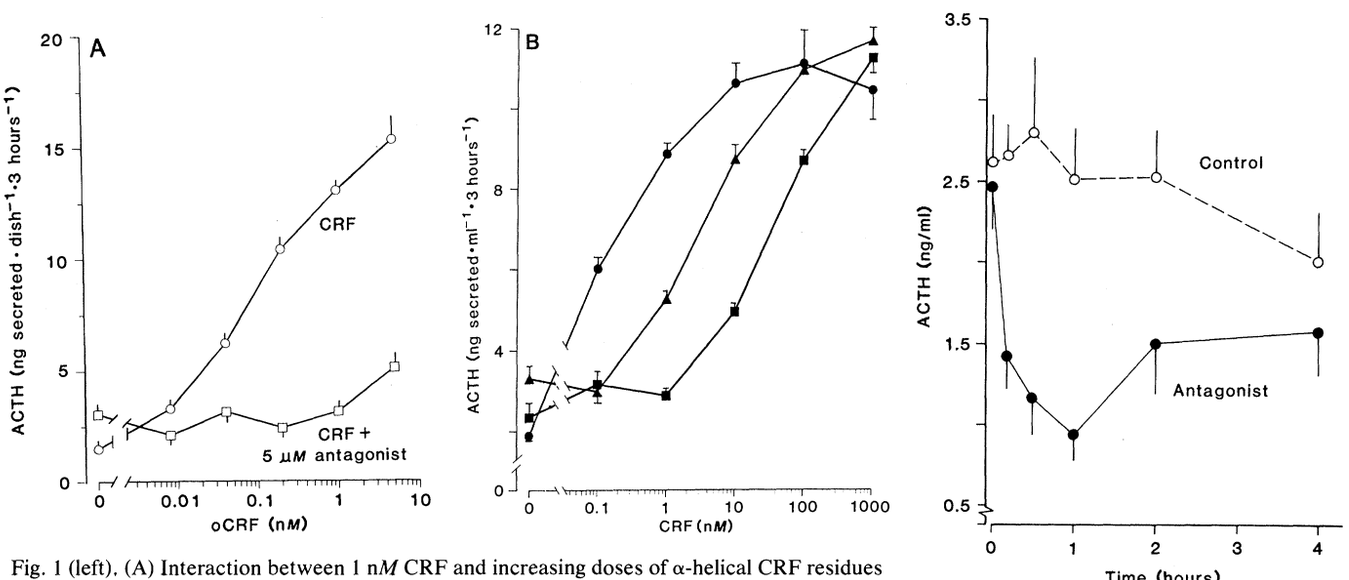

Fig. 1 (left). (A) Interaction between 1 n*M* CRF and increasing doses of α-helical CRF residues 9 to 41 on ACTH secretion by rat anterior pituitary cells in monolayer culture. Results are expressed as nanograms of ACTH secreted per tissue culture dish in 3 hours. (B) Effect of increasing doses of CRF on ACTH release in the presence of 500 n*M* or 5 μ*M* α-helical CRF (9 to 41). ●, Control; ▲, 500 n*M* α-helical CRF (9 to 41); ■, 5 μ*M* α-helical CRF (9 to 41). Fig. 2 (right). Effect of α-helical CRF residues 9 to 41 (antagonist) on ACTH release in nonanesthetized, adrenalectomized rats. Data represent mean ± S.E.M. (N = 6).

tive reverse phase high-performance liquid chromatography (HPLC) (*17*). Peptides were characterized by amino acid analysis, analytical HPLC under various conditions, and specific optical rotation.

On the basis of Chou and Fassman's data (*18*), Montecucchi and Gozzini (*19*) proposed that sauvagine and CRF assume a similar pattern of α-helixes and β-turns. We have presented spectroscopic and physicochemical evidence for such a secondary structure for oCRF, sauvagine, and U_1 (*20, 21*). We hypothesized that such a feature may be essential to receptor recognition and binding and by statistical analysis (*18*) designed and synthesized an analog that optimized α-helix formation by introduction of the amino acid with the highest P_α (*22*) value into areas where the aligned, naturally occurring members of the CRF family had nonidentical residues. In cases where the residues with the most α-helical–forming potential were unique, such as Glu^2 in rCRF and Leu^{12}, $Glu^{13,22}$, and Lys^{26} in sauvagine, the predominant substitution was introduced, that is, Gln^2, Phe^{12}, His^{13}, Ala^{22}, Gln^{26}. Exceptions were Ala^{32}, Leu^{36}, Glu^{39}, and Ala^{41}, because they increased α-helical–forming potential around a segment of the molecule which encompasses a proposed turn region (residues 32 to 37) or exhibited relatively high probabilities of both α-helix and β-sheet formation (residues 35 to 41) (Fig. 3). When assayed in rat pituitary cells in vitro, this analog was two to three times more potent than any of the members of the CRF family that were equally potent in releasing ACTH in vitro (*1*) (Table 1).

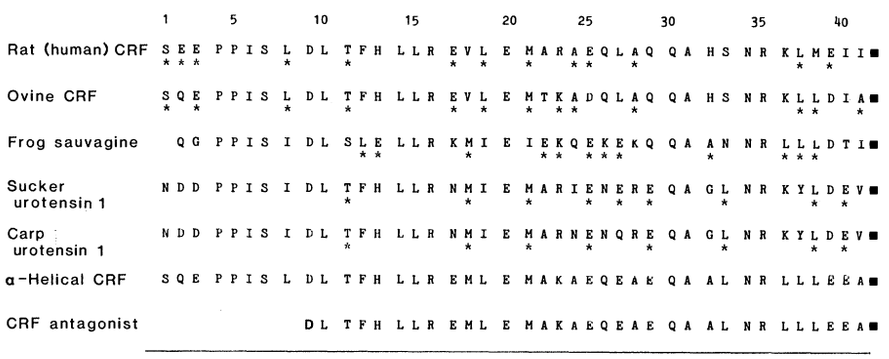

Fig. 3. The CRF family and antagonist structure. Boldface residues are those that also have been deleted to generate antagonists. Asterisks indicate amino acids with the largest P_α in the family.

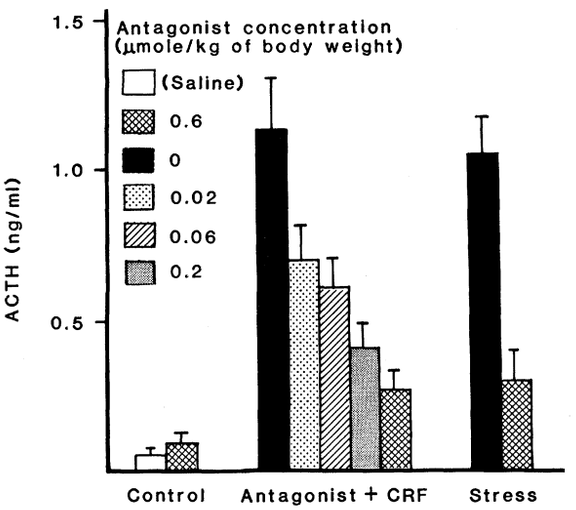

Fig. 4. Effect of α-helical CRF residues 9 to 41 (antagonist) on CRF- or stress-induced ACTH release in nonanesthetized, intact rats. The antagonist was administered intravenously and followed immediately by injection of CRF (0.45 nmole) or exposure to ether vapor for 3 minutes. Plasma ACTH concentrations were measured 5 minutes after injection of CRF and 10 minutes after exposure to ether. Bars represent mean ± S.E.M. (N = 8).

Table 1. Biological potencies of CRF analogs.

Analog	Agonist potency (range)	Intrinsic activity	Antagonist potency (range)
oCRF	1.0 (standard)	1.0	
oCRF (6–41)	0.11 (0.05 to 0.21)	1.0	
oCRF (7–41)	0.005	0.9	
oCRF (9–41)		< 0.1	0.15 (0.04 to 0.39)
α-Helical CRF	2.4 (1.7 to 3.5)	1.0	
α-Helical CRF			
(7–41)		0.5	0.17 (0.04 to 0.51)*
(8–41)		0.15	2.3 (1.15 to 5.1)
(9–41)		< 0.1	1.0 (standard)
(10–41)		< 0.1	0.71 (0.23 to 1.6)
[Leu^{12},Glu^{13}] α-Helical CRF			
(9–41)		< 0.1	0.91 (0.42 to 1.9)
[$Nle^{18,21}$] α-Helical CRF			
(8–41)		0.1	0.29 (0.12 to 0.63)
[$Nle^{18,21}$] α-Helical CRF			
(10–41)		0.13	0.35 (0.11 to 0.94)

*High level of intrinsic activity results in underestimation of antagonist potency of this analog.

Generation of an antagonist of small peptides has generally resulted from some specific amino acid substitution (often unnatural or of the D-configuration) (*13*) or deletion. Gonadotropin-releasing hormone (GnRH) (*11*) and human parathyroid hormone (PTH) exemplify the latter; with these, an antagonist could be generated by deleting His^2 in GnRH or two amino acids at the NH_2-terminus of the fully active fragment (residues 1 to 34) of PTH (*12*). We believed that the COOH-terminal amidated amino acid of CRF was important for biological activity and binding affinity, since des-Ala^{41}-oCRF had been shown to be a weak but full agonist. We therefore investigated the effects of systematic deletion of the NH_2-terminal amino acids of α-helical CRF on its biopotency in vitro. Most of the intrinsic activity was conserved even after deletion of residues 1 to 6 (Table 1). Deletion of the next three amino acids, however, generated peptides that showed definite partial agonism with low potency when tested in vitro and inhibited CRF-mediated release of ACTH when tested for antagonism. The potency of α-helical residues 9 to 41, used as a standard, was measured against the potency of several antagonists (Table 1); oCRF residues 9 to 41 could also antagonize the effects of CRF but with only 15 percent of the antagonist potency of the standard.

To confirm that neither aromatic residues Phe^{12} nor His^{13} (common to all sequences but that of sauvagine) was involved in the pituitary receptor activation process (as we had expected, since sauvagine was equipotent to oCRF in its

ability to release ACTH and β-endorphin), we synthesized [Leu[12], Glu[13]]α-helical CRF residues 9 to 41, which was found to be equipotent to the standard in its ability to inhibit oCRF-induced ACTH release in vitro (Table 1). To obtain more chemically stable antagonists, we also synthesized the Nle-substituted analogs at positions 18 and 21 of our α-helical CRF residues 8 to 41 and 10 to 41. These two analogs were found to be less potent than our standard.

The results of five independent experiments showed that the standard antagonist, α-helical CRF residues 9 to 41, blocked the secretion of ACTH that was stimulated by a 1-n*M* dose of CRF by 50 percent (197 ± 72 n*M*) (Fig. 1A). This inhibition was specific because the standard antagonist had no effect on the growth hormone–releasing factor–stimulated secretion of growth hormone, the gonadotropin-releasing factor–stimulated secretion of leuteinizing and follicle stimulating hormones, the thyrotropin-releasing factor–stimulated secretion of thyrotropin and prolactin, or the secretion of ACTH induced by another secretagog, phorbol myristate acetate. Several lines of evidence suggest that the antagonists acted by competing with CRF for binding to its receptors. The antagonists caused a parallel rightward shift in the CRF dose-response curves. Higher concentrations of CRF would completely overcome the blockade of the action of lower concentrations of CRF (Fig. 1B). The CRF antagonists could compete with an iodinated CRF analog for binding to anterior pituitary membranes (*23*).

The CRF antagonists were tested for their effects on the spontaneous release of ACTH in adrenalectomized rats. An intravenous injection of 1 mg of CRF antagonist (0.6 μmole per kilogram of body weight) caused a decrease in plasma ACTH amounts [measurements were performed as described (*1*)] that was statistically significant for 2 hours (Fig. 2). In the intact, nonanesthetized rats, the antagonist inhibited CRF-induced ACTH secretion in a dose-dependent manner that was significant at the 0.02-μmole dose (Fig. 4). This antagonist also prevented most, but not all, of the increase in ACTH caused by ether-exposure (Fig. 4).

These results indicate that administration of CRF antagonists reduces the spontaneous ACTH release observed after removal of the corticosteroid feedback, blocks the ACTH secretion caused by CRF, and inhibits most of the stressor-induced ACTH release in intact rats. These data are comparable to those obtained earlier in our laboratory with an antiserum to CRF (*2*) and further support the idea of a physiological role of endogenous CRF in regulating ACTH secretion. In addition, other studies (*24*) have shown that CRF antagonists can partially block the ether-exposure–induced activation of the sympathetic nervous system (*9*) and suggest a broader role for this neuropeptide in mediating the response to stressful stimuli.

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Genetic Influences in Criminal Convictions: Evidence from an Adoption Cohort

Abstract. *The possibility that genetic factors are among the causes of criminal behavior was tested by comparing court convictions of 14,427 adoptees with those of their biological and adoptive parents. A statistically significant correlation was found between the adoptees and their biological parents for convictions of property crimes. This was not true with respect to violent crimes. There was no statistically significant correlation between adoptee and adoptive parent court convictions. Siblings adopted separately into different homes tended to be concordant for convictions, especially if the shared biological father also had a record of criminal behavior.*

This study of the role of genetic factors in the etiology of criminal behavior is based on a register of 14,427 nonfamilial adoptions in a small northern European nation between 1927 and 1947. The register was established by a group of American and European investigators (*1*) and includes information on the adoptee and his or her adoptive and biological parents.

Court convictions were used as an index of criminal involvement. The data exclude minors below the age of 15, who are exempt from court convictions. Court records were obtained for all persons for whom data and place of birth were available (N = 65,516). The subjects' occupations permitted the coding of socioeconomic status (*2*).

Cases were excluded from the study if there was no record of place or date of birth, if the identity of the biological father could not be established, if the adoption was by a single woman, or if the birth date was prior to 1 January 1895. Exclusion of an adoptee resulted in exclusion of the entire adoptive family but, if a parent was excluded, the remaining subjects were retained for analysis. Data on individuals not fully identified are shown in Table 1.

Conviction rates of completely identified members of the adoptee families are also shown in Table 1. Rates for biologi-