Title
Inhibition of pituitary-adrenal secretion by a corticotropin releasing hormone antagonist in humans.

Journal
Molecular Psychiatry, 1(4)

ISSN
1359-4184

Authors
Baram, TZ
Mitchell, WG
Haden, E

Publication Date
1996-09-01

Published in final edited form as:
Mol Psychiatry. 1996 September ; 1(4): 320–324.

Inhibition of pituitary-adrenal secretion by a corticotropin releasing hormone antagonist in humans

TZ Baram[1], **WG Mitchell**[2], and **E Haden**[3]

[1] Departments of Pediatrics, Neurology and Anatomy & Neurobiology, University of California, Irvine, CA 92697-4475

[2]Division of Neurology, Childrens Hospital Los Angeles and University of Southern California, 4650 Sunset Boulevard, Los Angeles, CA 90027, USA

[3]Clinical Research Center, Childrens Hospital Los Angeles and University of Southern California, 4650 Sunset Boulevard, Los Angeles, CA 90027, USA

Abstract

Corticotropin releasing hormone (CRH) is the primary modulator of ACTH release from the pituitary, and a neuromodulator in limbic and autonomic brain regions. Dysfunction of CRH-mediated neurotransmission is emerging as a critical mechanism in several disorders. Therefore, modulation of CRH availability at receptor sites is a potentially powerful therapeutic tool. Inhibitory analogues of CRH have been tested in rodents and primates, but their safety and hormonal effects in humans are unknown. We administered a CRH-antagonist, α-helical-CRH-(9–41) to six individuals. Each received two intravenous infusions: 50 μg kg^{-1} on day 1, and 100 μg kg^{-1} on the following morning. These doses block both endocrine and central effects of CRH in experimental animals. ACTH, cortisol, electrolytes, glucose and autonomic parameters were monitored in comparison with control values. Infusion of CRH antagonist did not alter heart rate, blood pressure, temperature or plasma electrolytes and glucose. Pre-infusion plasma ACTH levels averaged 26.8 ± 6.7 pg ml^{-1} on day 1, and 29.0 ± 5.8 pg ml^{-1} on day 2. Post-infusion values were 11.8 ± 2 and 11.5 ± 2.4 pg ml^{-1}, significantly lower than pre-infusion levels. Plasma cortisol levels, which averaged 21.4 ± 4 μg dl^{-1} on the first morning and 22.9 ± 4.2 on the second, also decreased significantly after CRH antagonist infusions (to 14.0 ± 2.9 μg dl^{-1} on day 1, and 13.9 ± 3.0 μg dl^{-1} on day 2). Hormonal changes were transient, and circadian rhythm was not affected. Though not measured formally, euphoria, anxiety or somnolence were not observed. In conclusion, CRH antagonist administration to adults reduces hormonal secretion by pituitary corticotrophs, with resulting decrease in plasma ACTH and cortisol.

Keywords

CRF; hypothalamo-pituitary-adrenal; receptor; neuropeptide; ACTH; cortisol

Introduction

Corticotropin releasing hormone (CRH) is a neuropeptide with both neuroendocrine and neurotransmitter properties. The peptide, isolated originally from the hypothalamus[1] is the primary modulator of the release of ACTH from the pituitary in response to stress.[1–4] CRH functions as a neurotransmitter in a number of limbic and autonomic brain circuits.[5–9] Both

Correspondence: TZ Baram, Med Sci I, Zot 4475, UCI, Irvine, CA 92697-4475, USA.

the peptide and CRH receptors are widely but specifically distributed in the central nervous system (CNS).[2,10–14] For example, in the human brain, CRH is found in cortical interneurons of layers II and III while receptors predominate in layers I and IV.[15,16] Abnormal CRH levels in brain and spinal fluid, and of CRH receptors in the CNS, have been demonstrated in Alzheimer disease,[16] and abnormal CRH activity has been implicated in anxiety, depression and epilepsy.[3,16–18]

CRH neurons impinge on postsynaptic receptors.[11–14] Two members of the CRH receptor family are currently known, and consist of membrane-spanning G-protein-coupled molecules.[12–14] The first, CRF_1, is found in the CNS, immune cells and the pituitary, and is thus the candidate mediator of the endocrine effects of CRH. A second receptor, CRF_2, is found primarily in the CNS. Its unique distribution such as high levels in the ventral medial hypothalamus suggests a role in mediating the anorexic effects of CRH.[14]

A growing body of evidence (recently reviewed in Ref. 19) strongly implicates abnormal CRH-mediated neurotransmission in several prevalent human disorders. In the adult, ‘excessive’ CRH activity may play a causative role in anxiety and depression. During development, upregulation of CRH-mediated neuronal excitation may underlie some age-specific seizure disorders[18] and non-organic failure to thrive. Therefore, modulation of CRH-receptor activation offers powerful diagnostic and potentially therapeutic tools for a number of cardinal human disorders.[16–19] Inhibitory peptide analogues of CRH have been developed and tested in rodents and non-human primates.[20–22]

The goal of this study was to assess the safety and hormonal efficacy of the first and best characterized CRH-antagonist, alpha-helical CRH-(9–41), in normal human adults.

Material and methods

A phase I study was approved by the Food and Drug Administration (FDA, IND 45969), and by the institutional review board of Childrens Hospital Los Angeles (CHLA). Subjects were recruited via electronic mail advertisement, and consented to participate. Six individuals were enrolled, three men and three women, and are described in Table 1. Four were Caucasian, one African-American and one Asian. All were healthy, and on no anti-hypertensive or corticosteroid therapy (one woman was on contraceptives, a second took ibuprofen for premenstrual cramps).

Subjects were admitted to the Clinical Research Center (GCRC) at 7 am on the first study day, and infusions were carried out starting at 7.30–8.30 on two consecutive days. Vital signs and baseline plasma samples were obtained supine or sitting. Blood pressure, heart rate and core temperature were monitored at the onset of the infusions, and at 15-min intervals until their completion. These vital signs were measured hourly for the following 6 h. Subjects were fasted on both mornings until the end of each infusion, and permitted to ambulate 6 h after termination of the infusion. Plasma samples were also drawn 12 h after the end of the first infusion (evening nadir of ACTH, cortisol), and in the afternoon of the second day. Subjects were released on completion of a neurological examination performed 6–8 h after termination of the second infusion. Two (fasting) plasma samples, 2 h apart, were drawn on a follow-up visit, at 7.30 and 9.30 am. These were analyzed for ACTH and cortisol, to control for circadian fluctuations of these hormones.

The CRH antagonist, alpha-helical CRH-(9–41), was manufactured according to Good Manufacturing Practice by Bachem (Torrance, CA, USA). A sterile solution of 50 microgram (μg) per ml was infused over 2 h, to provide 50 μg kg^{-1} on the first study day, and 100 μg kg^{-1} on the second study day.

Plasma hormone levels were analyzed using commercial radioimmunoassays (Endocrine Sciences, Calabasas Hills, CA, USA). Sensitivity of ACTH assay was 5 pg ml^{-1}, and that of the cortisol assay was 1 μg dl^{-1}. Several samples were repeated, and interassay variabili ty averaged 8%. The significance of differences between groups was determined using the two-tailed paired Wilcoxon signed rank test without assumptions regarding value distribution.

Results

Infusion of CRH antagonist was not associated with significant alterations of blood pressure, heart rate or temperature (Table 1). Plasma glucose and electrolytes were also not affected.

Plasma ACTH and cortisol levels for individual subjects at the onset and at the completion of each infusion are shown in Figure 1. Prior to the first infusion, ACTH levels averaged 26.8 ± 6.7 pg ml^{-1}; mean plasma ACTH 2 h later, at the end of CRH antagonist infusion, was 11.8 ± 2 pg ml^{-1}. Cortisol levels at the end of the first infusion averaged 14.0 ± 2.9 μg dl^{-1}, compared with 21.4 ± 4.0 μg dl^{-1} at its onset. For both hormones, post-infusion values were significantly lower ($P < 0.05$) than pre-infusion ones. A similar reduction in ACTH and cortisol levels was evident with the second infusion: ACTH levels were 29 ± 5.8 and 11.5 ± 2.4 pg ml^{-1} prior to and after the infusion, respectively ($P = 0.06$); cortisol levels decreased from 22.9 ± 4.2 to 13.9 ± 3.0 μg dl^{-1} ($P = 0.03$). Plasma ACTH levels measured at 7.30 am on the follow-up morning averaged 20.4 ± 5.1 pg ml^{-1}. Two hours later (with no antagonist infusion), values for ACTH, 14.0 ± 3.1 pg ml^{-1}, were not significantly different ($P = 0.19$, Wilcoxon signed rank sum test). Corresponding cortisol levels were 29.2 ± 9.0 and 20.4 ± 7.6 μg dl^{-1}, with no significant difference between time-points.

Afternoon plasma ACTH levels (3–4 pm, 6 h after the second infusion) averaged 15.5 ± 2.7 pg ml^{-1}. The evening levels of plasma ACTH, measured 12 h post-infusion (9–10 pm) on the first study day averaged 8.8 ± 1.1 pg ml^{-1}. These values suggest that CRH antagonist infusion did not disrupt the circadian rhythm of this hormone. Concomitant cortisol levels, 15.1 ± 3.0 μg dl^{-1} in the afternoon and 5.9 ± 1.6 μg dl^{-1} at night, were also consistent with a preserved circadian fluctuation.

Formal cognitive and emotional testing of the subjects was not a goal of this study. However, subjects were monitored continuously during infusions, engaged in conversation and encouraged to express concerns and feeling. No overt differences in behavior or cognition were noted between pre- and post-infusion interviews. All subjects had normal neurological examinations—including a Mini-Mental Status battery and measures of balance, coordination and dexterity—prior to discharge.

Discussion

CRH itself has been given to humans, and has profound effects on autonomic functions and hormonal out put.[17,21–23] CRH elevates plasma ACTH and cortisol, increases the respiratory drive,[22] and results in hypotension and flushing.[23] Currently, the clinical uses of CRH are limited to diagnosis, and, rarely, to respiratory stimulation. CRH antagonists should be expected to exert effects secondary to unavailability of CRH at its receptors. Outside the CNS, lower ACTH and cortisol, and potentially, secondary effects of the lack of these hormones (such as hypoglycemia, electrolyte imbalance) may be expected. Although transient reductions of ACTH and cortisol were observed at the end of CRH antagonist infusion, no secondary effects on blood pressure, glucose or electrolytes were observed in this study.

The doses used in this study (50 and 100 μg kg^{-1}), were clearly sufficient to block pituitary CRH receptors and decrease plasma ACTH and, in turn, cortisol levels. On both days, post-

infusion reductions of hormone levels were statistically significant. As infusions were given in the morning, at time of diurnal peak of ACTH and cortisol levels, it may be argued that the reduction simply represents the normal circadian fluctuation. However, when samples were obtained from subjects at the same hours but without alpha-helical CRH-(9–41) infusion, no significant reductions of plasma ACTH and cortisol levels were evident. This is consistent with a direct role for the antagonist in decreasing ACTH release. Further, an 'artificial' elevation of pre-infusion ACTH and cortisol levels due to the stress of hospitalization and blood drawing is unlikely. On the second morning of the study, subjects were comfortable and relaxed, and samples were drawn via an existing catheter.

No obvious anxiolytic or euphoric effects of alpha-helical CRH-(9–41) infusion were observed. The antagonist is a peptide, and is not expected to penetrate the intact blood-brain barrier. However, alpha-helical CRH (9–41) interacts avidly with the plasma CRH-binding protein, and active uptake of the complex cannot be excluded. The scope of this study precluded formal assessment of potential CNS effects of CRH antagonist infusion. Recent placebo-controlled studies have revealed placebo effects in up to 40% of subjects for measures of 'dizziness' or 'anxiety'.[24] Subjects in this study were hospitalized and subjected to the stressful procedures of intravenous line insertion and blood drawing. Furthermore, placebo infusions were not administered. Therefore, any conclusions regarding cognitive effects of the CRH antagonist in this study would be tentative.

CRH-dysfunction outside the CNS may underlie some forms of Cushing's syndrome and immuno-inflammatory abnormalities.[3,21] Nevertheless, the majority of disorders in which CRH dysregulation may play a key role are those affecting the brain, such as depression, anorexia nervosa, anxiety and some developmental epilepsies.[3,17–19,25] Therefore, therapeutic use of CRH receptor blockers should require compounds that can cross the blood–brain barrier and alter the availability of CRH at receptor sites in the CNS. The cloning of two members of the CRH receptor family[12–14] has permitted the development of screening methods for non-peptide compounds which should be able to penetrate the blood–brain barrier and reach critical CRH receptors in limbic and cortical brain regions. The alterations of ACTH and cortisol levels seen in this study, using relatively low doses of a CRH antagonist, suggest that depression of pituitary-adrenal function poses a potentially important side effect of CRH antagonists considered for clinical research trials for neurological and psychiatric disorders.

In summary, the administration of an inhibitory analogue of CRH to humans is reported. The lack of observed side effects, in conjunction with evidence of efficacy of the antagonist on hormonal parameters, provides strong incentive for developing CRH antagonists as therapeutic agents in several critically important human disorders.

Acknowledgments

Supported by an Innovative Clinical Research grant of the CHLA Research Institute (TZB), and by the NIB NCRR GCRCgrant MO 1 RR-43. TZB was supported in part by NIH NS 28912. The authors are grateful to Drs R Parkman, K Weinberg and IT Lott for their encouragement, to Dr J Pech and the CHLA pharmacy, and to Linda Schultz and Sam Alkass for their assistance.

References

1. Vale W, Rivier C, Brown MR, Spiess J, Koob G, Swanson L, Bilezikjian L, Bloom F, Rivier J. Chemical and biological characterization of corticotropin releasing factor. Rec Prog Horm Res. 1983; 39:245–270. [PubMed: 6314446]
2. Sawchenko PE, Imaki T, Potter E, Kovacs K, Imaki J, Vale W. The functional neuroanatomy of corticotropin releasing factor. CIBA Foundation Symp. 1993; 172:5–21.

3. Tsigos C, Chrousos GP. Physiology of the hypothalamic pituitary adrenal axis in health and dysregulation in psychiatric and autoimmune disorders. Endocrinol Metabol Clin North Am. 1994; 23:451–466.
4. Makino S, Smith MA, Gold PW. Increased expression of CRH and vasopressin mRNA in the hypothalamic PVN during repeated stress: association with reduction of glucocorticoid receptor mRNA levels. Endocrinology. 1995; 136:3299–3309. [PubMed: 7628364]
5. Fox EA, Gruol DL. CRF suppresses the afterhyperpolarization in cerebellar Purkinje neurons. Neurosci Lett. 1993; 49:103–107. [PubMed: 8469370]
6. Ehlers CL, Henriksen SJ, Wang M, Rivier J, Vale W, Bloom FE. Corticotropin releasing factor produces increases in brain excitability and convulsive seizures in rats. Brain Res. 1983; 278:332–336. [PubMed: 6605787]
7. Baram TZ, Schultz L. CRH is a rapid and potent convulsant in the infant rat. Dev Brain Res. 1991; 61:97–101. [PubMed: 1914160]
8. Rainnie DG, Fernhout BJ, Shinnick-Gallagher P. Differential actions of corticotropin releasing factor on basolateral and central amygdaloid neurons *in vitro*. J Pharmacal Exper Therap. 1992; 263:846–858.
9. Curtis AL, Pavcovich D, Grigoriadis DE, Valentino RJ. Previous stress alters corticotropin releasing factor neurotransmission in the locus ceruleus. Neuroscience. 1995; 65:541–550. [PubMed: 7777167]
10. Young, WS, III. Distribution and regulation of CRF-mRNA in brain using *in situ* hybridization histochemistry.. In: De Souza, EB.; Nemeroff, CB., editors. Corticotropin Releasing Factor: Basic and Clinical Studies of a Neuropeptide. CRC; Boca Raton: 1990. p. 213-220.
11. De Souza, EB.; Kuhar, MJ. Corticotropin-releasing factor receptors: autoradiographic identification.. In: Martin, JB.; Barchas, JD., editors. Neuropeptides in Neurologic and Psychiatric Disease. Raven Press; New York: 1986. p. 179-198.
12. Wong ML, Licinio J, Gold PW. Localization of CRH receptor mRNA in adult rat by *in situ* hybridization. Endocrinology. 1994; 135:2275–2278. [PubMed: 7956950]
13. Potter E, Sutton S, Donaldson C, Chen R, Perrin M, Lewis K, Sawchenko PE, Vale WW. Distribution of CRF-receptor mRNA expression in the rat brain and pituitary. Proc Natl Acad Sci. 1994; 91:8777–8781. [PubMed: 8090722]
14. Lovenberg TW, Liaw CW, Grigoriadis DE, Clevenger W, Chalmers DT, De Souza EB, Oltersdorf T. Cloning and characterization of functionally distinct corticotropin-releasing factor receptor subtype from rat brain. Proc Natl Acad Sci. 1995; 92:836–840. [PubMed: 7846062]
15. Bissette G, Reynolds GP, Kilts CD, Widerlov E, Nemeroff CB. Corticotropin releasing factor like immunoreactivity in senile dementia of the Alzheimer type. JAMA. 1985; 254:3067–3069. [PubMed: 3877182]
16. Behan DP, Heinrichs SC, Troncoso JC, Liu XJ, Kawas CH, Ling N, De Souza EB. Displacement of corticotropin releasing factor from its binding protein as a possible treatment for Alzheimer's disease. Nature. 1995; 378:284–287. [PubMed: 7477348]
17. Nemeroff CB. New vistas in neuropeptide research in neuropsychiatry: focus on corticotropin releasing factor. Neuropsychopharmacology. 1992; 6:69–75. [PubMed: 1610487]
18. Baram TZ. Pathophysiology of massive infantile spasms: perspective on the role of the brain adrenal axis. Ann Neurol. 1993; 33:231–237. [PubMed: 8388675]
19. Lightman SL. Corticotropin releasing factor: from stress to cognition. Nature. 1995; 378:233–234. [PubMed: 7477335]
20. Rivier J, Rivier C, Vale W. Synthetic competitive antagonists of corticotropin releasing factor: effect on ACTH secretion in the rat. Science. 1984; 224:889–891. [PubMed: 6326264]
21. Nieman LK, Chrousos GP, Oldfield EH. The ovine corticotropin releasing hormone stimulation test and the dexamethasone suppression test in the differential diagnosis of Cushing disease. Ann Int Med. 1986; 105:862–867. [PubMed: 3022629]
22. Nink M, Salomon E, Coutinho M, Treese N, Bernhard G, Krause D, Beyer J, Lehnert H. Corticotropin releasing hormone is a respiratory stimulant in humans. Life Sci. 1994; 23:1793–1799. [PubMed: 8196493]

23. Kubler A, Rothacher G, Knappertz VA, Kramer G, Nink M, Beyer J, Lehnert H. Intra- and extracerebral blood flow changes and flushing after intravenous injection of human corticotropin releasing hormone. Clin Invest. 1994; 72:331–336.

24. Summary of clinical studies. Lamotrigine package insert, Glaxo-Wellcome. Oct. 1995

25. Licinio J, Wong K-L, Gold PW. The hypothalamic-pituitary-adrenal axis in anorexia nervosa. Psychiat Res. 1996; 62:75–83.

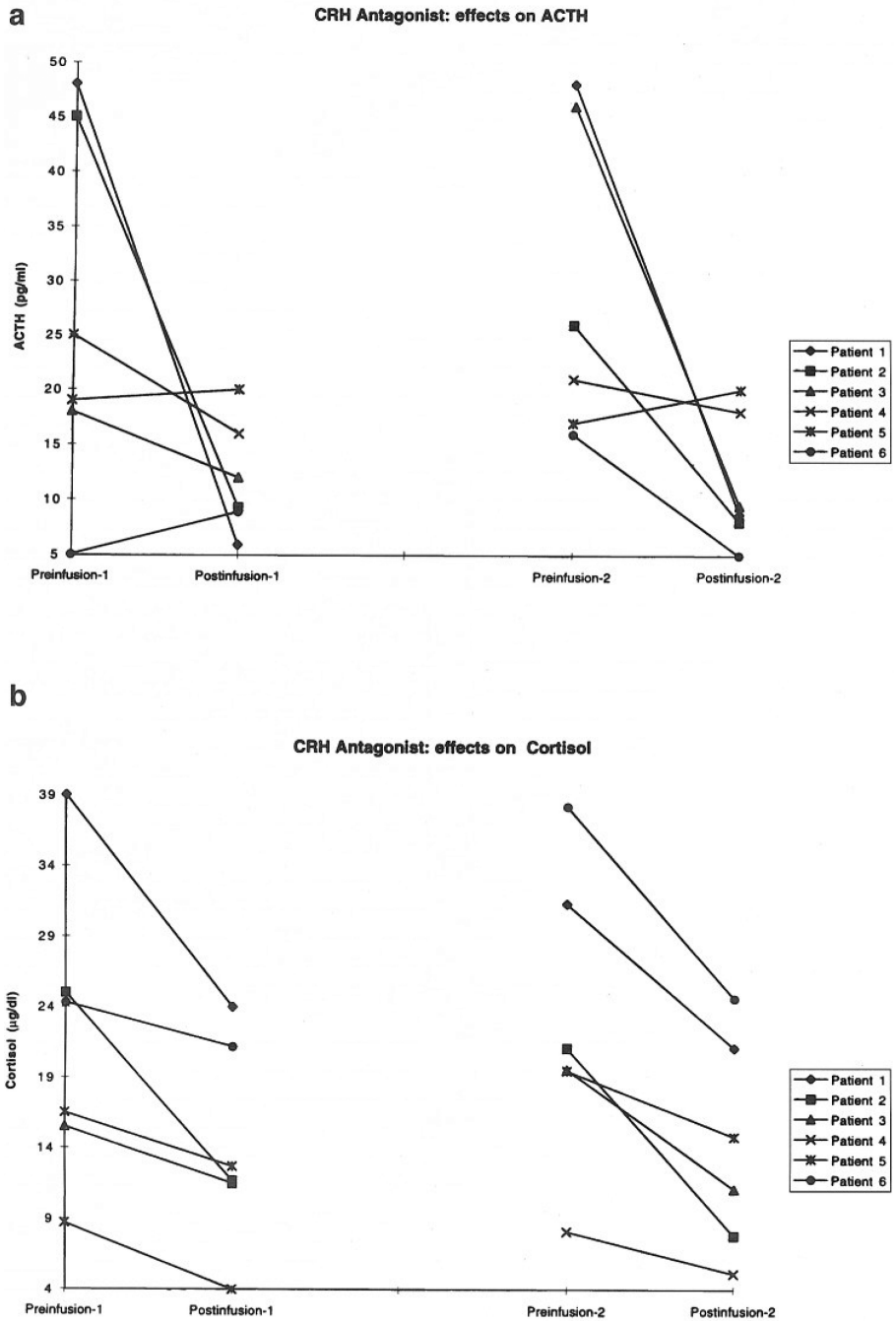

Figure 1.

Plasma levels of ACTH (a) and cortisol (b) in each individual subject before and immediately after infusions of alpha-helical CRH (9–41). The first infusion provided 50 μg kg^{-1} and the second 100 μg kg^{-1} of the CRH antagonist. Hormones were measured by radioimmunoassay as described in the text.

Table 1

Characteristics of study subjects

No.	Age /sex	Ethnicity	Medications	Preinfusion			During infusion[a]		
				BP[b]	HR	Temp	BP	HR	Temp
1	30/F	Caucasian	None	107/58	52	36.5	97/67–108/68	50–60	36–37
2	29/M	Caucasian	None	138/77	70	37.4	113/65–135/82	60–81	37–37,4
3	24/M	Caucasian	None	144/64	62	36	102/53–122/74	50–61	36–36.8
4	37/F	Af/Amer[c]	Ibuprofen	121/76	7a	37.3	106/71–126/77	73–81	36.7–37.2
5	19/M	Caucasian	None	136/69	60	36	114/59–129/77	52–65	36–36.7
6	28/F	Japanese	BCP[d]	143/102	68	37,1	133/94–152/110	66–79	36.6–37.2

[a] Parameters were measured every 5–15 min; the range for each subject is provided.

[b] BP = blood pressure; HR = heart rate; Temp = oral temperature (°C).

[c] Af/Ainer = African-American.

[d] BCP = birth control pill.