

Blunted ACTH and Cortisol Responses to Systemic Injection of Corticotropin-Releasing Hormone (CRH) in Fibromyalgia

Role of Somatostatin and CRH-Binding Protein

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ABSTRACT: Thirteen female patients suffering from fibromyalgia (FM) and thirteen female age-matched controls were intravenously injected with a bolus dose of 100 µg corticotropin-releasing hormone (CRH), and the evoked secretion pattern of ACTH, cortisol, somatostatin, and growth hormone (GH) was followed up for two hours, together with the plasma levels of CRH. The increases of ACTH and cortisol following CRH were not significantly different between controls and FM patients. The increase of plasma CRH following its injection was significantly higher in FM patients and lasted about 45 min, paralleled by an increase of somatostatin with a similar time course. Basal GH levels were significantly lower in FM patients. GH increased in FM patients 90 min after injection of CRH, coincident with decreasing CRH and somatostatin levels, while GH levels in controls rather decreased with the lowest values occurring 90 min after CRH. The results support the concept that the hormonal secretion pattern frequently observed in FM patients is primarily caused by CRH, possibly as a response to chronic pain and stress. The elevated levels of CRH in the circulation of FM patients suggest elevated levels of CRH-binding protein, which could explain why the levels of ACTH and cortisol between controls and FM following CRH do not differ.

KEYWORDS: ACTH; cortisol; corticotropin-releasing hormone; fibromyalgia; somatostatin; CRH-binding protein

INTRODUCTION

Though the most prominent symptom of fibromyalgia (FM) is pain of the musculoskeletal system, characteristic hormonal deviations and many psychological symptoms point to a primary disturbance originating most likely within the central nervous system (CNS).^{1,2} FM is predominantly found in women. It has been

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proposed that FM should be considered a "stress-related syndrome" because symptoms often have their onset triggered by stress, whether psychological, infectious, or traumatic.³⁻⁵ Stress exerts its effect via as yet unknown central pathways that stimulate the hypothalamus to release multiple ACTH secretagogues, corticotropin-releasing hormone (CRH) and antidiuretic hormone being the most important.^{6,7} The elevated activity of CRH neurons seems to alter the setpoint also of other hormonal axes, because the regulation of many hormonal axes is severely disturbed in FM. FM patients resemble most frequently symptoms of hypothyroidism^{8,9} and suppression of the hypothalamic-pituitary-gonadal axis.¹⁰ Lowered levels of insulin-like growth factor I (IGF-I) in FM have suggested a disturbance in the regulation of growth hormone (GH).¹¹ The present study was performed to explore whether systemic injection of CRH causes in FM patients an altered release of somatostatin and whether this correlates with their lowered values of GH.

PATIENTS AND METHODS

We studied the plasma levels of CRH, ACTH, cortisol, GH, and somatostatin in 13 female patients (all of whom met the criteria for FM in accordance of the classification of the American College of Rheumatology)¹² and in 13 healthy age-matched controls, following an intravenous (i.v.) bolus injection of 100 µg of CRH. The mean age of FM patients was 49.0 ± 1.8 years, and that of the controls was 50.1 ± 1.8 years. No significant difference of body mass index between the two groups was found. Patients and control subjects taking antidepressants, tranquilizers, neuroleptics, or hormones were requested to stop drug intake at least one week, and analgesics one day, before participating in the study. None of the subjects suffered from an inflammatory rheumatic disease or any other circulatory, hormonal, or psychiatric disease. In controls and patients, at 09:00 A.M., an i.v. catheter for blood sampling was inserted and the first blood sample was taken, and the second sample was taken exactly one hour later. Thereafter, patients and controls received i.v. within 60 seconds 100 µg of CRH (Ferring, Kiel, FRG). Test samples were drawn after 15, 30, 45, 60, 90, and 120 minutes. Blood was collected in chilled tubes containing EDTA, trasyolol, and mercaptoethanol. Samples were kept on ice, immediately centrifuged at 4°C, and stored at -70°C until evaluation. CRH and somatostatin were measured after extraction of the peptides with buffered 60% acetonitrile from C₁₈ silica columns (Supelclean LC-18, Supelco, Bellefonte, PA, USA), using enzyme immunoassay (EIA) kits (Phoenix Pharmaceuticals, Mountain View, CA, USA). ACTH was measured with an enzyme-linked immunosorbent kit (DRG International, Marburg, FRG). Human GH was detected using an immunoradiometric assay; cortisol, using an EIA Kit (BioChem, Freiburg, FRG).

Statistical Analysis

Each data point is reported as a mean \pm SEM. Statistical analysis was carried out by the use of standard one- and two-way parametric analysis of variance (ANOVA) with repeated measures design coupled with paired Student's *t*-test or the Wilcoxon matched-pairs signed-ranks test using Sigmastat software (Jandel Scientific, Corte Madera, 1995). A value of $p < 0.05$ was taken to indicate a significant difference.

RESULTS

The CRH values of FM patients differed from controls in that they were elevated under basal conditions and rose to significantly higher levels following the systemic injection of CRH, the differences between FM and controls lasting for 60 min. We found that the pre-stimulation values of somatostatin of FM patients were likewise significantly higher, and that the injection of CRH was followed by significantly higher values. A prominent finding was the occurrence of significantly lower basal values of GH in FM patients, values that were not significantly altered by the injection of CRH until 60 min. Thereafter occurred a rise of GH in FM patients, while the values of controls significantly decreased below pre-stimulation levels (see FIGURE 1 and TABLE 1). The prestimulation values of ACTH of FM patients and controls were not significantly different, nor were the increases following CRH different from group to group. Maximal values of ACTH were reached 45 min after CRH (TABLE 1). The pre-stimulation values of cortisol were significantly lower in FM patients. After injection of CRH, cortisol rose to maximal values within 60 min, the hormonal profiles of the two groups showing no significant differences (TABLE 1).

DISCUSSION

The hormonal pattern specifically characterizing FM comprises a multitude of deviations. These deviations include an altered reactivity of the hypothalamic–pituitary–adrenal axis, lowered thyroid hormone levels combined with a blunted response of thyroid-stimulating hormone (TSH), an exaggerated response of prolactin to thyrotropin-releasing hormone (TRH), lowered levels of IGF-I, and a blunted response of luteinizing hormone (LH) to luteinizing hormone-releasing hormone (LHRH).^{3–5,8–11,13–15} These findings, which were obtained in numerous laboratories, support the conclusion that the observed hormonal deviations constitute an entity that conforms specifically to FM. It is still an open question whether the perturbations occurring in the various hormonal axes are causally involved in eliciting FM or whether the distortion of the hormonal profile is a reaction of the CNS to the main symptom of FM, which is musculoskeletal pain. Therefore, pain has been considered to act as the main stressor in FM causing the activation of CRH neurons. CRH is the key physiological mediator of the endocrine, autonomic, and behavioral responses to stress. The central pathways that are engaged in the transmission of stress signals that stimulate the hypothalamus to release the multiple ACTH secretagogues are as yet largely unknown. It has been proposed that any kind of stress or nociceptive signals converge, after being perceived by the cortex and assembled by the limbic system, onto hypothalamic CRH neurons as the common final pathway. Since the limbic system exerts both excitatory and inhibitory effects on CRH neurons, in stress, and in FM in particular, either the excitatory pathways prevail or the inhibitory pathways are being suppressed.

Apart from stimulating the pituitary ACTH-producing cells, CRH affects various other neuronal systems and even peripheral tissues. CRH has been shown to stimulate in hypothalamic and cortical neurons somatostatin secretion.^{16,17} Hypothalamic somatostatin, secreted into the hypophyseal portal vessels and transported to the

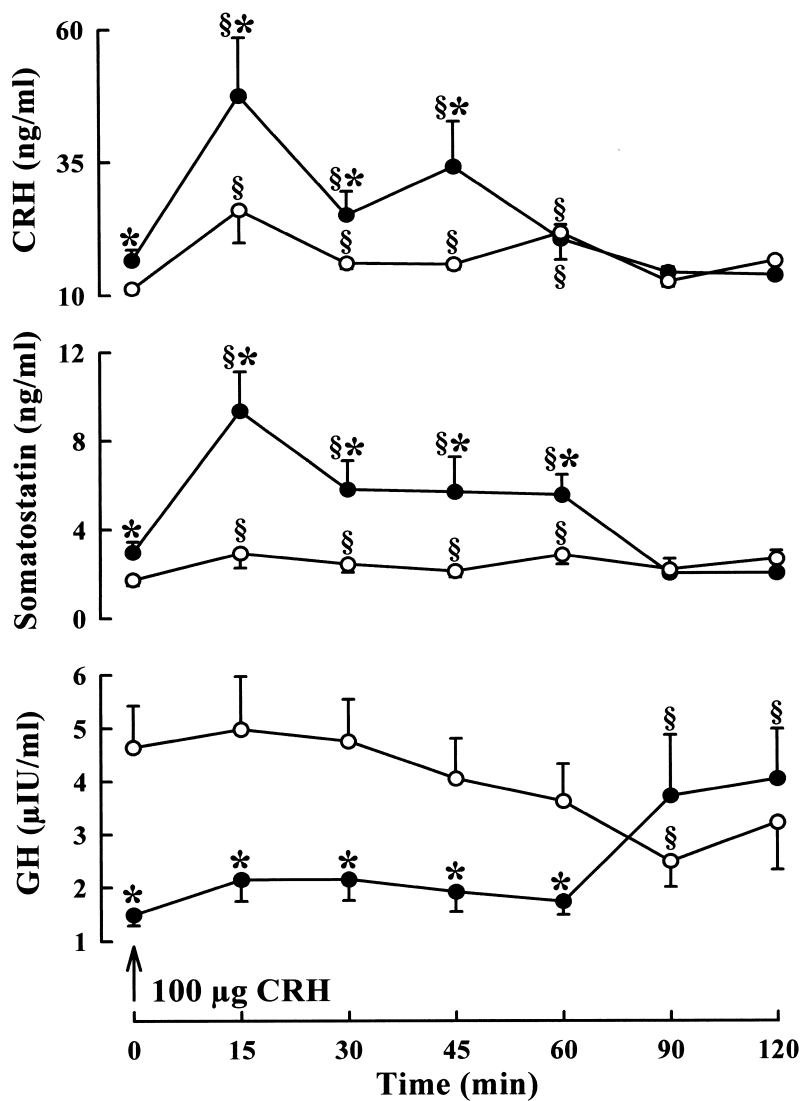


FIGURE 1. Effects of bolus injection of 100 µg of CRH on plasma values of CRH, somatostatin (SOM), and GH of 13 controls and 13 FM patients. Each value is a mean \pm SEM. ○, controls; ●, FM patients; §significant differences from prestimulation values; *significant differences between control group and FM patients ($p < 0.05$).

TABLE 1. Effects of intravenous bolus injection of 100 µg of CRH on plasma values of CRH, somatostatin (SOM), GH, ACTH, and cortisol among 13 controls and 13 FM patients

Time	0 min	15 min	30 min	45 min	60 min	90 min	120 min	
CRH (ng/ml)	Controls	11.2 ± 0.9	25.9 ± 6.1 ^a	15.8 ± 1.0 ^a	15.5 ± 0.9 ^a	21.4 ± 5.1 ^a	12.2 ± 1.0	16.0 ± 2.3
	FM	16.6 ± 2.0 ^b	47.4 ± 10.9 ^{ab}	24.9 ± 4.4 ^{ab}	33.9 ± 8.5 ^{ab}	20.2 ± 2.7 ^a	13.9 ± 1.0	13.4 ± 0.7
SOM (ng/ml)	Controls	1.7 ± 0.3	3.0 ± 0.7 ^a	2.4 ± 0.4 ^a	2.1 ± 0.3 ^a	2.8 ± 0.4 ^a	2.2 ± 0.5	2.6 ± 0.4
	FM	3.0 ± 0.5 ^b	9.3 ± 1.8 ^{ab}	5.8 ± 1.3 ^{ab}	5.7 ± 1.6 ^{ab}	5.5 ± 0.9 ^{ab}	2.0 ± 0.1	2.0 ± 0.1
GH (µU/ml)	Controls	4.6 ± 0.8	5.0 ± 1.0	4.8 ± 0.8	4.1 ± 0.8	3.6 ± 0.7	2.5 ± 0.5 ^a	3.2 ± 0.9
	FM	1.5 ± 0.2 ^b	2.2 ± 0.4 ^b	2.2 ± 0.4 ^b	1.9 ± 0.4 ^b	1.7 ± 0.3 ^b	3.7 ± 1.2 ^a	4.1 ± 0.9 ^a
ACTH (pg/ml)	Controls	14.3 ± 1.6	26.0 ± 4.5 ^a	26.2 ± 3.3 ^a	26.5 ± 3.8 ^a	24.3 ± 3.0 ^a	19.2 ± 1.4	16.4 ± 1.6
	FM	14.8 ± 0.7	25.1 ± 1.4 ^a	27.5 ± 1.3 ^a	28.8 ± 1.3 ^a	26.9 ± 1.3 ^a	21.3 ± 0.5 ^a	19.7 ± 0.7
Cortisol (ng/ml)	Controls	103 ± 16	144 ± 15 ^a	188 ± 13 ^a	207 ± 13 ^a	216 ± 12 ^a	195 ± 12 ^a	168 ± 11 ^a
	FM	67 ± 7 ^b	123 ± 13 ^a	168 ± 15 ^a	186 ± 19 ^a	203 ± 18 ^a	186 ± 16 ^a	164 ± 14 ^a

NOTE: Each value is a mean ± SEM.

^a Significant differences from prestimulation values.

^b Significant differences between control group and FM patients (*p* < 0.05).

anterior pituitary gland, physiologically inhibits the release of GH and TSH. The decline of circulating GH and TSH levels in stress, and FM, has been assumed therefore as being mediated by somatostatin.^{10,18–22}

The present study shows that in FM, pre-stimulation levels of somatostatin are elevated and that systemically injected CRH increases somatostatin secretion to a significantly higher extent. Both the CRH and somatostatin profiles closely mirror the plasma levels of GH, in that the basal values of GH are lowered in FM, and remain low as long as somatostatin levels are elevated. One could conclude from these results that the sensitivity of the somatotrophic cells of the pituitary to somatostatin in FM is not altered. The reason why GH hormone levels are lowered in FM in the present study may therefore be related primarily to the higher CRH levels. In contrast, the different levels of CRH observed in the two groups of the present study do not elicit different ACTH and cortisol values. It has to be elucidated whether the higher levels of somatostatin in FM contributed to the blunted ACTH response to CRH, as they might suggest a direct inhibitory action of somatostatin on pituitary adrenocorticotropes.^{23,24}

Under the assumption that we extracted the concentrations of both free and bound CRH, then the blunted response of ACTH and cortisol to CRH could indicate, on the other hand, that FM patients had an elevated plasma capacity to bind CRH. CRH mediates its endocrine effect via two classes of CRH receptors. Once released into the circulation, CRH is transported by a CRH-binding protein (CRH-BP),²⁵ which binds CRH with an affinity equal to or greater than that of the CRH receptors. CRH-BP has been found to block CRH-mediated ACTH release, and it has been hypothesized that CRH-BP plays an important *in vivo* modulatory role by regulating levels of “free” CRH and other CRH-like peptides in the pituitary and in the CNS by showing that it can reduce or block CRH-mediated ACTH secretion from corticotropes.²⁶ The differentiated effects of CRH in the present study in stimulating effectively the hypothalamus to release somatostatin on the one hand, and the blunted effect on pituitary ACTH release on the other, may indicate that CRH-BP is not able to cross the blood–brain barrier or that CRH is more efficiently cleaved from its binding protein within the CNS.

In male mice, CRH-BP is expressed in corticotropes, the pituitary CRH target sites, to neutralize the biological activity of CRH. Elevated levels of pituitary steady-state CRH-BP mRNA have been found in female versus male mice, with three-fold higher levels during proestrus than diestrus.²⁷ Plasma levels of CRH-BP are higher in women than men, but this is unrelated to circulating estrogen levels. Dexamethasone treatment lowers CRH-BP in all subjects, and it has been found, in Cushing’s patients, that an iv bolus of 100 micrograms of human CRH further lowers plasma CRH-BP at 15 min, disclosing a positive feedback on CRH levels.²⁸ Circulating levels of CRH-BP decrease considerably in the last trimester of pregnancy of healthy women, resulting in further elevation of bioavailable plasma CRH associated with an increase in ACTH, which parallels that of CRH.²⁹ Whether a decrease of CRH-BP occurs also in pregnancy of FM patients has to be elucidated, since there is a general worsening of FM symptoms during pregnancy, with the last trimester experienced as the worst period.³⁰ An altered expression of CRH-BP apparently exerts a profound impact on the biological activity of CRH, which mediates by acting on CRH

receptor 1 (CRHr1) anxiety-related behavior,³¹ but also non-opioid-mediated antinociception,^{32,33} and may thus modulate many clinical disorders.

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