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Corticotropin releasing hormone (CRH) response in patients with early rheumatoid arthritis due to polymorphisms in the CRH gene

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ABSTRACT

Objective. *To further evaluate the impact of corticotropin releasing hormone (CRH) promoter polymorphisms on the stress response in rheumatoid arthritis (RA) patients an insulin hypoglycemia test (IHT) was performed studying the dynamics of CRH production.*

Methods. *Polymorphisms of the human CRH promoter were determined in controls and cortisol naive patients with early RA. Serum glucose and plasma CRH were measured at baseline and up to 120 min following induction of hypoglycemia.*

Results. *During IHT RA patients bearing the A2B2 allele exhibited an earlier CRH response compared to A1B1 positive patients.*

Conclusion. *Stress-induced response of CRH is differentially modulated by CRH promoter polymorphisms in RA patients.*

Introduction

From animal models of arthritis as well as from clinical studies in rheumatoid arthritis (RA) patients a central role of the hypothalamic-pituitary-adrenal (HPA) axis in onset and severity of RA was suggested (1-3). Corticotropin-releasing hormone (CRH) is the strongest known activator of the HPA axis which has been functionally implicated in endocrine-immune responses (4).

We described several polymorphisms in the highly conserved regulatory region of the CRH gene which is located on chromosome 8q13 (5). Three polymorphisms co-segregated absolutely resulted in two alleles *A1* and *A2* with a further polymorphism being assigned as alleles *B1* and *B2*. Population investigations demonstrated a distortion in the distribution of the resulting compound alleles A1B1, A2B1 and A2B2 between RA patients and healthy controls (6). Furthermore, we have shown that polymorphisms of the CRH gene modulate significantly the reactivity of the HPA axis *in vitro* (7). Recently, it could be demonstrated that CRH polymorphisms modulate basal cortisol levels and cortisol/ACTH ratio in RA patients (8). To further evaluate the impact of CRH promoter polymorphisms on the activity of the HPA axis in RA patients we studied plasma CRH levels under basal conditions and hypoglycemia-induced stress.

Material and methods

We studied 18 patients with early RA being glucocorticoid naïve (disease duration <9 months, disease activity score (DAS) ≥3.2; 15 women, 3 men; mean age 53.5±9.3 years) and 8 healthy controls from the same regional area in Germany. All subjects gave informed consent and the study was approved by the ethics committee of our university.

In controls and RA patients the A1B1, A2B1 and A2B2 polymorphisms of the human CRH promoter were determined utilising restriction fragment length polymorphism of PCR amplified DNA products of the CRH promoter as described earlier (5).

Insulin hypoglycemia test (IHT) was performed by i.v. application of weight adjusted insulin (0.1 IU/kg, Sanofi Aventis GmbH, Germany). Blood glucose and serum CRH levels were measured at baseline and up to 120 min following induction of hypoglycemia in 30 min intervals as described earlier (9).

Results

Studying the CRH promoter polymorphisms revealed a greater genetic variability for RA patients: 12 patients and all control subjects had the A1B1 compound allele. 5 RA patients were A2B2 positive while 1 patient exhibited the A2B1 allele. All patients were diagnosed with early RA and were glucocorticoid-naïve taking NSAR only. For statistical reasons, further analyses were performed with the A1B1- and the A2B2-positive individuals. Stratifying the RA patients for the CRH polymorphisms revealed a significant difference in CRP levels (A1B1 35.4±8.7 mg/l *vs.* A2B2 11.2±3.2 mg/l; $p<0.05$). However, there was no significant difference for disease activity (DAS28 in A1B1 4.3±0.3 *vs.* A2B2 4.4±0.6).

The IHT was validated in all subjects since all of them achieved a serum glucose <2.2 mmol/l (<40 mg/dl) and showed mild clinical symptoms of hypoglycemia (tachycardia, sweating). There was a significant difference of blood glucose levels between the con-

trol group (A1B1) and both RA groups (A1B1, A2B2): at base line as well as at time points 15 min, 90 min and 120 min glucose levels were significantly lower in controls compared to both RA subgroups (p<0.01-0.02) (Fig. 1). The integrated glucose level expressed as area under the curve (AUC) was significantly higher in A1B1 patients compared to A1B1 controls (p<0.05) (Table I).
After development of hypoglycemia, the CRH peak was significantly higher at 30 min in A2B2 patients compared to A1B1 RA patients (p<0.01) and the control group (p<0.02) (70.8±8.13, 55.0±3.8 and. 56.3±3.94 pg/ml, respectively) (Fig. 2). In addition, CRH levels peaked later in A1B1 patients (at 60 to 90 min) and the control group (Fig. 2). Interestingly, CRH AUC was significantly higher in A2B2 patients compared to A1B1 controls (Table I).

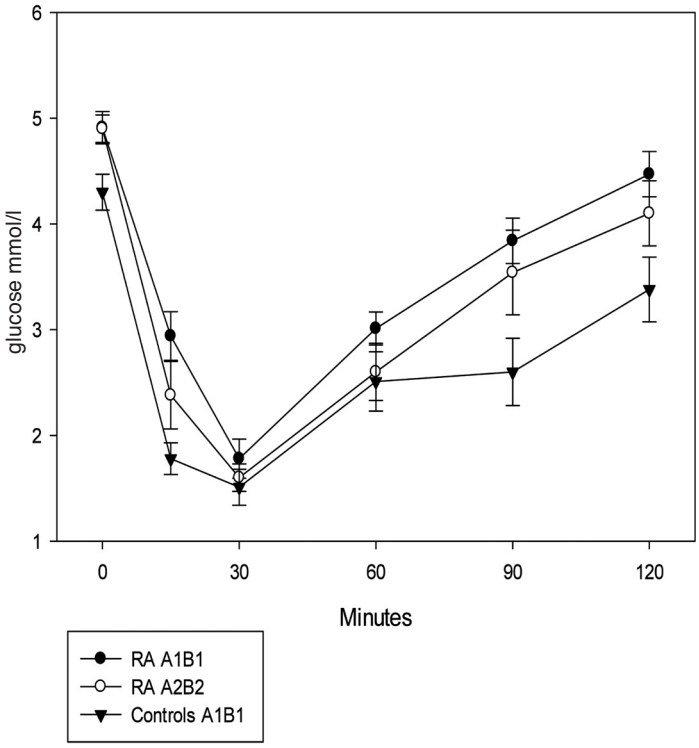

Fig. 1. Changes in blood glucose levels after insulin-induced hypoglycemia in RA patients with A1B1 (n=12) and A2B2 (n=5) CRH promoter polymorphisms compared to healthy controls (A1B1; n=8.)

Table I. Integrated response expressed as area under the curve (AUC) for glucose and corticotropin releasing hormone (CRH) in rheumatoid arthritis patients and healthy controls.

AUC	Rheumatoid arthritis		Healthy controls	p-value <0.05
	A1B1=12	A2B2=5	A1B1=8	
Glucose mmol/l	394 ± 35.6	360.45 ± 31.7	298 ± 26.5	A1B1 RA *vs.* A1B1 controls
CRH pg/ml	7132 ± 568.4	7410 ± 343.2	6560 ± 389.3	A2B2 RA *vs.* A1B1 controls

The results of the IHT were given as mean ± standard error of the mean (SEM) and were analysed by SigmaStat (SYSTAT Software Inc., Chicago, IL, USA). Comparisons of the results were carried out by One Way ANOVA. All pairwise multiple comparison procedures were calculated by Holm-Sidak method with a significance level of $p \leq 0.05$ to reduce the α-error.

Discussion

Our findings provide the first evidence that the stress-induced response of CRH is differentially modulated by CRH promoter polymorphisms in RA patients. CRH polymorphisms exhibited a significant influence on the response of CRH upon hypoglycemia with A2B2 positive RA patients reacting quicker and producing higher levels of CRH.
CRH is produced in the hypothalamus regulating the HPA axis, although it has been found in various tissues, including inflammatory sites (10). Due to the short plasma half-life of CRH (four minutes) it is assumed that the contribution of hypothalamic CRH to the total plasma CRH is small (11). However, under certain circumstances, such as the IHT, hypothalamic CRH release leads to measurable increments in plasma CRH concentrations. Furthermore, the significant differences we found point to an influence of the CRH polymorphisms on the hypothalamic CRH production. Together with our previous results we can conclude that the peripherally rather than centrally produced CRH is influenced by the CRH promoter polymorphisms due to more pronounced differences in glucocorticoid production (8). Of interest, there was a significant difference for CRP levels between the patient groups indicating a relationship between CRH promoter polymorphisms, HPA axis reactivity and inflammation in RA patients. However, there was no difference between the patient groups for the DAS28 and it remains to be clarified whether the lower CRP levels in A2B2 positive RA patients is consequence of the polymorphism. Interestingly, it was shown that the CRH A2 allele was significantly increased in patients with seronegative RA of late onset (12). Therefore, it can be hypothesised that patients bearing the A2B2 allele exhibit an increased stress-induced HPA axis function that is more sufficient to inhibit ongoing inflammation and to delay the onset of disease. Due to the low frequency of the rare CRH allele we were not able to perform an IHT in healthy controls or other chronic inflammatory diseases. Hence, it remains to be clarified whether the A2B2-induced modulation of the HPA axis is specific for RA or a general phenomenon. Moreover, it has to be clarified how the CRH polymorphisms interact with the stress response in RA patients with longstanding disease often requiring long-term glucocorticoid therapy. Firstly, one could speculate that a different CRH reactivity may lead to a modulation of HPA axis suppression under these circumstances. Secondly, it has to be taken into account that the chronic disease itself exerts an influence on the HPA axis reactivity (13-14).
Blood glucose levels were significantly higher in RA patients at base line as

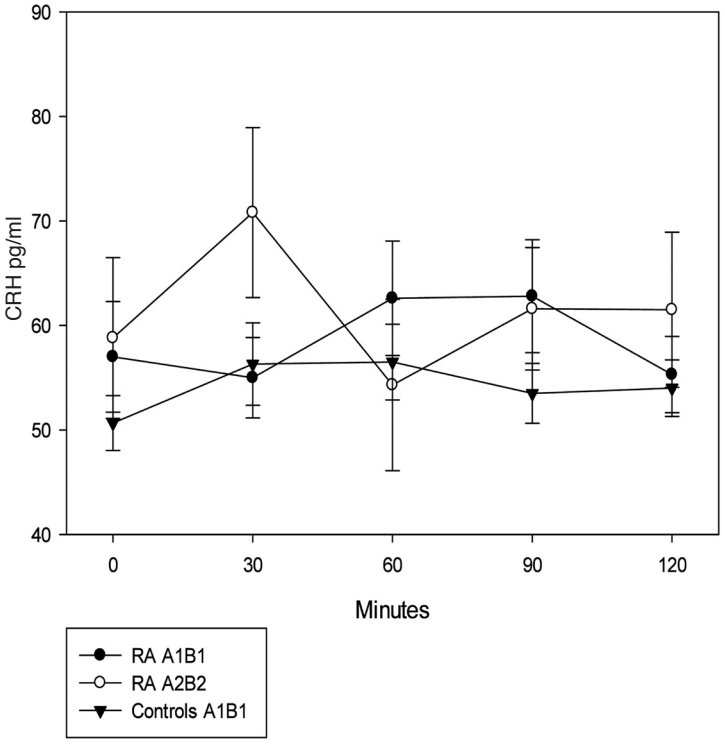

Fig. 2. Changes in plasma CRH levels after insulin-induced hypoglycemia in RA patients with A1B1 (n=12) and A2B2 (n=5) CRH promoter polymorphisms compared to A1B1 healthy controls (n=8).

well as at several time points during the IHT. In particular between A1B1 positive RA patients and controls the difference was significant. Therefore, CRH polymorphisms might contribute to decreased insulin sensitivity and impaired glucose handling being described in RA patients with active disease (15). On the other hand, patients with the A2B2 polymorphism developed significantly quicker hypoglycemic blood glucose levels compared to patients with the A1B1 polymorphism. However, the pathophysiologic relevance of altered glucose metabolism is uncertain but may reflect a possible link with increased risk of atherosclerotic cardiovascular disease in RA (16). Of interest, regulation of the HPA axis via CRH receptors (CRH-R2) and CRH binding protein is considered to be a potent regulator of cardiovascular adaptations to stress (17-18). However, we did not study the concentration of CRH binding protein nor did we investigate haemoglobin A1C as a marker for glucose metabolism.

Taken together, our results demonstrate for the first time that in RA patients promoter polymorphisms in the CRH gene modulate CRH reactivity upon stress and possibly glucose metabolism as well. Due to the low numbers of subjects studied in this pilot study the mechanism and the impact of CRH polymorphisms need to be further characterised with respect to clinical relevance and possible new therapeutic avenues.

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