European Journal of Endocrinology (2008) **159** 649–652 ISSN 0804-4643

CLINICAL STUDY

Cortisol response to ACTH stimulation correlates with blood interleukin 6 concentration in healthy humans

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Abstract

Objective: Interleukin 6 (IL6) has the ability to influence each level of the hypothalamo-pituitary–adrenocortical (HPA) axis. The aim of the study was to test whether IL6 concentration correlates with the adrenal cortex response to ACTH in healthy humans. We postulated that higher basal IL6 concentration would be associated with the higher cortisol response to the stimulation.

Design and methods: Basal IL6 concentration was measured and a low dose (1 μg) ACTH test was performed to assess cortisol response. Twenty-seven apparently healthy subjects (11 male, 16 female, mean age 31.1 years, age range 22–47 years) were included in the study.

Results: Data are presented as mean ± S.E.M. Basal IL6 level was 0.84 ± 0.10 pg/ml. Basal cortisol was 351.9 ± 18.3 nmol/l. Maximal cortisol during synacthen test was 653.0 ± 20.6 nmol/l. Maximal cortisol increment was 301.1 ± 20.0 nmol/l. IL6 concentration was not correlated with basal or maximal cortisol concentration, but correlated significantly with cortisol increment ($r = 0.63$, 95% confidence interval) 0.42–0.83).

Conclusions: In our study, we found that higher basal IL6 concentration is associated with the higher cortisol response to ACTH stimulation. Based on previous research and our data, IL6, even in low concentrations and under physiologic conditions, modulates adrenal cortex responsivity to ACTH. Therefore, it seems that immune modulation of HPA axis is also present under physiologic and not only pathologic conditions.

European Journal of Endocrinology **159** 649–652

Introduction

It is well known that interleukin 6 (IL6) has the ability to activate the hypothalamo-pituitary–adrenocortical (HPA) axis influencing each level of the axis. IL6 stimulates corticotrophin-releasing hormone (CRH) release from the hypothalamus of rats in a dose-dependent manner (1, 2). IL6 also directly stimulates corticotrophs, even in CRH knockout animals (3, 4). In humans, acute administration of IL6 increases adrenocorticotropin (ACTH) and cortisol concentrations (5). Also, a dose-dependent increase in ACTH and cortisol is seen in response to IL6 administration (6). IL6 also acts directly on the adrenal cortex, stimulating glucocorticoid release. However, in the absence of ACTH, IL6 fails to elicit glucocorticoid response. Consequently, ACTH is a necessary permissive factor, enabling direct cytokine actions on the adrenal gland (7).

As IL6 augments ACTH stimulation of the adrenal cortex, higher IL6 levels should be correlated with increased cortisol response to ACTH stimulation. In order to test this hypothesis, we performed a low dose (1 μg) synacthen test and measured basal IL6 concentration in healthy, non-stressed humans. Previous studies used stress (infection or exercise), or direct infusion of IL6 to assess its effects on HPA axis. However, we decided to analyse the influence of non-stimulated IL6 level and to vary ACTH concentration.

Materials and methods

Subjects

Twenty-seven apparently healthy subjects (11 male, 16 female, mean age 31.1 years, age range 22–47 years, mean body mass index (BMI) 23.5 kg/m^2, BMI range 17.1–28.9 kg/m^2) were included in the study. Inclusion criteria were: i) normal renal and liver function tests, ii) no intercurrent disease in the last three weeks, iii) no signs of endocrine dysfunction, iv) not taking any drugs, v) normal C-reactive protein (CRP) level and vi) normal arterial blood pressure. Subjects were instructed to have a good rest and at least 8 h sleep before the test. Ethical approval was obtained, and all participants gave informed consent.

DOI: 10.1530/EJE-08-0544
Online version via www.eje-online.org

Study protocol

After a twelve-hour fast, blood was sampled for cortisol determination. An indwelling venous catheter was placed in the antecubital vein 30 min before the start of the test. The low dose synacthen test started at 0800 h with the i.v. injection of 1 μg synacthen (Novartis Pharma Schweiz). Blood samples for cortisol determination were taken from the i.v. cannula at 0, 15 and 30 min. The blood sample for IL6 determination was taken at 0 min. All blood samples were immediately separated and kept frozen at −80 °C until assayed.

As previously described, for the low dose test a vial of 250 μg synacthen was diluted in normal saline solution to a concentration of 0.5 μg/ml (8). The solution was used immediately.

Assays

IL6 was determined using enzyme immunoassay technique (Quantikine HS, R&D Systems Inc., Minneapolis, MN, USA) with intra- and interassay coefficient of variation (CV) of 6.9–7.4 and 6.5–9.6% respectively. Cortisol was determined using a RIA (Cort-CT2, CIS bio international, Gif-Sur-Yvette CEDEX, France) with intra- and interassay CV of 3.8–4.6 and 4.4–5.5% respectively.

Statistical analysis

Data are presented as mean ± S.E.M. To compare cortisol and IL6 between sexes a *t*-test was used. To assess correlation a Pearson correlation was used. For statistical analysis R, version 2.7.0 was used (9). To obtain correlation confidence intervals (CI), the simpleboot package was used (10). Bootstrap CI calculations were based on 10 000 bootstrap replicates. To compare IL6 versus cortisol regression slopes, the nlme package was used (11).

Results

Basal IL6 level was 0.84 ± 0.10 pg/ml (0.75 ± 0.10 pg/ml male, 0.89 ± 0.14 pg/ml female). Basal cortisol was 351.9 ± 18.3 nmol/l (351.9 ± 24.5 nmol/l male, 329.7 ± 25.0 nmol/l female). Maximal cortisol during the synacthen test was 653.0 ± 20.6 nmol/l (666.5 ± 29.4 nmol/l male, 643.8 ± 28.8 nmol/l female). Maximal cortisol increment (Δcortisol) was 301.1 ± 20.0 nmol/l (282.3 ± 30.4 nmol/l male, 314.1 ± 26.9 nmol/l female). There was no difference between sexes in any of the parameters. IL6 concentration was not correlated with basal or maximal cortisol concentration. However, IL6 concentration was significantly correlated with cortisol increment (all: $r=0.63$, 95% CI 0.42–0.83, $P<0.01$, male: $r=0.74$, 95% CI 0.23–1.00, $P=0.01$, female: $r=0.56$, 95% CI 0.28–0.81, $P=0.02$, Fig. 1).

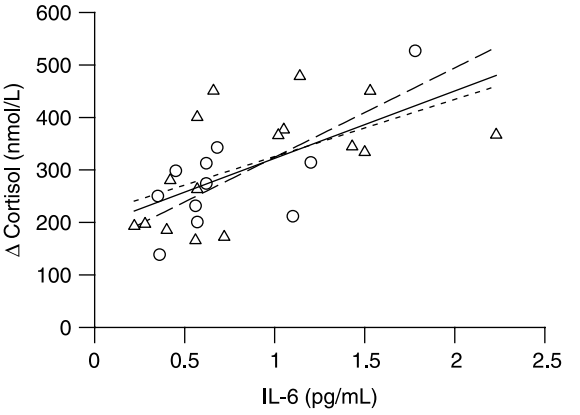

Figure 1 Relation between IL6 and Δcortisol. Circles represent males and triangles females. Regression lines are drawn and represent all subjects (solid line), males (dashed line) and females (dotted line).

Slopes of the IL6 versus cortisol increment regression lines did not differ between males and females (Fig. 1).

Discussion

The aim of the study was to test whether adrenal cortex response to ACTH in healthy humans correlates with blood IL6 concentration. We postulated that higher basal IL6 concentration would be associated with the higher cortisol response to the stimulation. We decided not to manipulate IL6 concentration, as IL6 influences each level of the HPA axis. Instead, we used a low (1 μg) ACTH test to assess the reactivity of the adrenal cortex.

In addition to the endocrine system, the neural and immune systems are also involved in adrenal gland regulation (12). Dissociation between central activation of the HPA axis and the adrenal cortex can occur in a variety of situations (13). In septic patients, a few days after an insult, ACTH levels fall, but cortisol levels stay high (14). In *Trypanosoma cruzi* infected mice there is an increase in corticosterone, not accompanied by the increase in ACTH (15).

IL6 is an important stimulator of the HPA axis. It has the ability to influence each level of the HPA axis. IL6 induces CRH release from the hypothalamus of rats in a dose-dependent manner (1, 2). In CRH knockout animals, IL6 stimulates ACTH release, presumably by binding to its own receptor on corticotrophs (3). In humans, s.c. IL6 administration induced a dose-dependent increase in ACTH and cortisol (6). IL6 also acts directly on the adrenal cortex, stimulating glucocorticoid release. Silverman *et al.*, in an elegant experiment, showed that ACTH is a necessary permissive factor enabling direct IL6 action on the adrenal gland. In the presence of IL6, corticosterone response was increased in wild-type mice and in CRH knockout mice. However, in hypophysectomized animals cortisol

response was absent (7). In humans, high frequency sampling showed that significant cross correlation between IL6 and cortisol exists, IL6 preceding cortisol. This was present both in physiologic and pathologic conditions (16, 17).

In our study, we found that a relatively low plasma IL6 concentration (0.84 ± 0.10 pg/ml) in healthy humans, modulates adrenal cortex response to ACTH stimulation. Although IL6 concentration was not correlated with the basal cortisol, it was significantly correlated with the maximal cortisol increment (Δcortisol, $r=0.63$, 95% CI 0.42–0.83 $P<0.01$).

Therefore, subjects with higher plasma IL6 concentration had higher cortisol response to ACTH stimulation. This implies that, even under physiologic conditions, IL6 modulates adrenal cortex response to ACTH stimulation.

The major problem with this study is that this is a correlation study. It is well known that spurious correlations are possible. Some other factor e.g. IL1, could influence the adrenal cortex (18). But in this case, those factors would also be correlated with IL6. Also, a large body of previously published data argues that the influence of IL6 on cortisol secretion is significant. Another possibility is false correlation due to an unusual data distribution. However, bootstrap CI shows that the obtained correlation is reliable, and not caused by data distribution. Furthermore, dividing the data by sex and analysing regression line slopes, showed that slopes in males and females are the same, arguing against unusual data distribution as a cause of IL6 and cortisol increment correlation.

IL6 receptors are present in the adrenal cortex of many species, including humans (3, 19–21). It has been shown that IL6 alone stimulates glucocorticoid release from primary cultures of rat, bovine and human adrenal cells (19–23). However, in order to stimulate glucocorticoid release, a long incubation time (>12 h) is required. IL6 also acts in synergy with ACTH, and in the presence of ACTH IL6 increases glucocorticoid release even at times when, alone, it is ineffective (19, 22). In mice ACTH is a necessary permissive factor that enables IL6 to stimulate glucocorticoid secretion, at least during the first 36 h of viral infection (7). In both humans and animals, during inflammatory response or IL6 treatment, dissociation between central activation of the HPA axis and the adrenal cortex secretion is found (14, 24–27).

In humans IL6 increases under different conditions, such as exercise or sleep deprivation (17, 28). It was shown that an increase in cytokines is associated with insulin resistance and visceral obesity (29). Therefore, it was proposed that IL6 is involved in the response to somatic stress and in the development of obesity and metabolic syndrome (16, 29). In obesity, there is a dissociation between ACTH and cortisol secretion, which is pronounced in the evening, (30) at approximately the same time the second zenith of IL6 concentration occurs (31). Therefore, an IL6 induced increase in cortisol secretion could be a part of the stress response and could be involved in the development of obesity, metabolic syndrome, fatigue and sleep disturbances (16, 29). Our data support these hypotheses.

In our study, we found that a higher basal IL6 concentration is associated with the higher cortisol response to ACTH stimulation. Based on previous research and our data, IL6, even in low concentrations and in physiologic conditions modulates adrenal cortex responsivity to ACTH. Therefore, it seems that immune modulation of HPA axis is present under physiologic and not only pathologic conditions.

Declaration of interest

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

Funding

This work was supported by a grant from the Ministry of Science and Environmental Protection of the Republic of Serbia (145031).

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Received 18 August 2008
Accepted 22 August 2008