The endotoxin-induced increase of cytokines is followed by an increase of cortisol relative to dehydroepiandrosterone (DHEA) in healthy male subjects

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Abstract

Dehydroepiandrosterone (DHEA) and DHEA sulphate (DHEAS) inhibit T-helper lymphocyte type 2 immune reactions and exert anti-inflammatory effects in some chronic inflammatory diseases. Both DHEA and, in particular, DHEAS levels are dramatically decreased in chronic inflammatory diseases whereas cortisol levels remain stable or are elevated. However, the time course of cortisol relative to DHEA production is not known. We tested whether administration of endotoxin to healthy male subjects can induce an early predominance of cortisol relative to DHEA and DHEAS. It is demonstrated that endotoxin induces a dose-dependent increase of cortisol in relation to DHEA (no effect at 0.2 ng endotoxin/kg body weight (b.w.), clear effect at 0.4 and 0.8 ng/kg b.w., $P < 0.05$) and DHEAS (tested at 0.4 ng/kg b.w., $P = 0.014$). The increase of cortisol relative to DHEA

appears 4 h after endotoxin injection and 2 h after a strong increase of interleukin (IL)-6 relative to tumour necrosis factor (TNF). In addition, an increase of cortisol relative to 17OH-progesterone was observed. The ratio of serum IL-6/TNF was positively correlated with the ratio of serum cortisol/DHEA ($R_{\text{Rank}} = 0.472$, $P = 0.041$) and serum cortisol/17OH-progesterone ($R_{\text{Rank}} = 0.514$, $P = 0.048$). In conclusion, dissociation of cortisol relative to DHEA, DHEAS or 17OH-progesterone appears very early during a systemic inflammatory response which is associated with an increase of IL-6 relative to TNF. As in chronic inflammatory diseases, during an acute inflammatory response with endotoxin, these physiological hormone changes are probably necessary to achieve adequate cortisol levels at the expense of adrenal androgens.

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Introduction

Dehydroepiandrosterone (DHEA) has been shown to inhibit proinflammatory cytokines such as secretion of tumour necrosis factor (TNF) (Danenberg *et al.* 1992, Di Santo *et al.* 1996, Araghi-Niknam *et al.* 1997, Kimura *et al.* 1998, Padgett & Loria 1998) and interleukin (IL)-6 (Daynes *et al.* 1993, Straub *et al.* 1998a, Gordon *et al.* 2001). On the other hand, DHEA seems to favour T-helper lymphocyte type (Th) 1 reactions whereas DHEA inhibits Th2 immune responses (Wilder 1996a). DHEA may exert an anti-inflammatory effect in Th2-driven diseases (van Vollenhoven *et al.* 1995, Andus *et al.* 2000) and a more proinflammatory effect in Th1-driven diseases. Thus, in some diseases, decreased serum hormone levels of DHEA and DHEA sulphate (DHEAS) may be unfavourable.

DHEAS is dramatically decreased in most chronic inflammatory diseases but this phenomenon is still unexplained (Masi *et al.* 1984, Sambrook *et al.* 1988,

Hedman *et al.* 1992, Hall *et al.* 1993, Nilsson *et al.* 1994, de la Torre *et al.* 1995, Mateo *et al.* 1995, Wilder 1996b, Formiga *et al.* 1997, Straub *et al.* 1998b, Cutolo *et al.* 1999). As expected, prior glucocorticoid treatment further decreases DHEAS, which has been demonstrated in several diseases (Sambrook *et al.* 1988, Hedman *et al.* 1992, Hall *et al.* 1993, Straub *et al.* 1998b). Low levels of DHEAS have also been demonstrated after short-term inflammatory states such as acute cholestasis (Zietz *et al.* 2001a). Thus, there seems to be an early inflammation-dependent deterioration of androgen production in the adrenal glands.

In this study, we wanted to investigate whether low-dose endotoxaemia by administration of lipopolysaccharide (LPS) of *Salmonella abortus equi* in healthy male volunteers leads to an early predominance of cortisol in relation to DHEA or DHEAS. The prominent increase of IL-6 and TNF may be the most important factor for this phenomenon (Michie *et al.* 1988, Mastorakos *et al.* 1993, Pollmächer *et al.* 1993, Späth-Schwalbe *et al.* 1994, Tilders *et al.* 1994). Thus, we measured these two important

cytokines in order to shed light on their possible role for the dissociation of cortisol relative to DHEA, DHEAS or 17OH-progesterone.

Subjects, Materials and Methods

Experimental subjects

The experimental procedure was approved by the Ethics Committee for Human Experimentation at the Max-Planck-Institute of Psychiatry. In the dose-response study, 17 healthy male subjects (mean age 25.8 years; range 21–33 years) and in the single-dose study 24 healthy male subjects (mean age: 27.4 years, range 21–35 years) participated after having given written informed consent. These healthy men were subjects of earlier studies that investigated different aspects of endotoxin injection (Schuld *et al.* 2000, Mullington *et al.* 2000, Haack *et al.* 2001). All subjects were screened by medical history, physical examination, laboratory investigations, electrocardiogram and electroencephalogram to exclude acute and chronic illness.

Endotoxin preparation

A standardised sterile preparation of *Salmonella abortus equi* endotoxin was used which was essentially free of protein and nucleic acids (see Galanos *et al.* (1979) for details of preparation and properties).

Experimental procedure

At 1630 h, the subjects entered the laboratory and were under continuous observation during the entire experiment. At 1700 h, an i.v. canula was inserted into an antecubital vein. The blood line was kept patent with saline solution containing heparin. One lead electrocardiogram and temperature were monitored throughout the entire experiment. At 1800 h, subjects received a light meal, and later only mineral water was offered *ad libitum*. At 2300 h, light was turned off and the subjects were instructed to sleep until they woke up spontaneously in the next morning. They were offered breakfast 30 min after awakening but remained in bed until 1200 h the next day. Blood was sampled from 2300 h every 2 h until 0900 h the next morning (3 ml serum and 3 ml plasma; sum = 18 ml serum and 18 ml plasma) while subjects were sleeping. Sampled blood was stabilised with Na-EDTA (1 mg/ml blood) and aprotinin (300 KIU/ml blood), and following immediate centrifugation adequate aliquots were stored at -20°C or -80°C . At 2300 h, 0.9% saline as a control vehicle or endotoxin was i.v. injected in a single-blind, placebo-controlled fashion. In the dose-response study 0.2 ($n=7$), 0.4 ($n=5$) and 0.8 ($n=5$) ng endotoxin per kg body weight (b.w.) were administered. Due to ethical reasons, these doses induce cytokine maxima that are clearly lower

as compared with acute infectious diseases. In the single-dose study, only 0.4 ng endotoxin/kg b.w. was injected ($n=12$ endotoxin, $n=12$ placebo, cross-over design 14 days later). The groups did not differ in mean age or body mass index.

Laboratory parameters

We used radioimmunoassays for the quantitative determination of serum levels of cortisol (Coulter Immunotech, Marseilles, France; detection limit: 10 nmol/l). Serum levels of 17OH-progesterone (IBL, Hamburg, Germany; detection limit: 0.3 nmol/l), DHEAS (IBL; detection limit: 130 nmol/l), DHEA (Diagnostic Systems Laboratory, Webster, Texas; detection limit: 0.13 nmol/l), IL-6 (high sensitivity Quantikine, R&D Systems, Minneapolis, MN, USA; detection limit: 0.2 pg/ml) and TNF (high sensitivity Quantikine, R&D Systems; detection limit: 0.2 pg/ml) were measured by means of immunoassays. Intraassay and interassay coefficients of variation were below 10% in each test.

Statistical analysis

In order to compare means in two different groups the Mann-Whitney signed rank test was used (SPSS/PC for Windows, V.10.0.5, SPSS, Inc., Chicago, IL, USA). Regression analysis was done by Spearman rank correlation analysis (SPSS) and linear regression lines are demonstrated in the Figures. Time-related changes of serum IL-6 concentration within the control group during the night was detected by the non-parametric Friedman test for numerous dependent values (SPSS). In this Friedman test, $P<0.05$ indicates a significant change of the variable during the test procedure, which demonstrates an increase over time. P -values of less than 0.05 were considered to be significant and means are always given $\pm$ S.E.M.

Results

Serum levels of adrenal hormones during the endotoxin test

Four hours after injection of 0.4 ng endotoxin/kg b.w., we observed a significant increase of serum levels of cortisol as compared with control conditions (Fig. 1A). During the following time, serum levels of cortisol under control conditions also increased and, thus, no difference was detected between 0500 and 0900 h (Fig. 1A). No marked differences were observed for serum levels of 17OH-progesterone, DHEA and DHEAS (Fig. 1B,C,D).

Serum levels of cytokines during the 0.4 ng/kg endotoxin test

As demonstrated in Fig. 2, there was a marked increase of serum TNF and serum IL-6 with a maximum at 2 h after

Figure 1 Time course of adrenal steroid hormones after i.v. endotoxin injection in healthy male volunteers. Serum levels of cortisol (A), 17OH-progesterone (B), DHEA (C) and DHEAS (D) are depicted in nmol/l. Filled symbols represent data $\pm$ S.E.M. of subjects under control conditions, and open symbols demonstrate serum levels of subjects after i.v. injection of 0.4 ng endotoxin per kg b.w. The rectangle in (A) demonstrates the dissociation of the two curves in patients with/without prior endotoxin administration.

endotoxin injection (0100 h). In addition, under control conditions, subjects demonstrated a small early morning rise of serum IL-6 ($P=0.002$ in the Friedman test, Fig. 2B). Furthermore, control subjects demonstrated elevated levels of TNF in relation to IL-6 2 h after endotoxin injection (Fig. 2C), which was the opposite 6 h after endotoxin injection (Fig. 2C). Thus, under endotoxin conditions, this ratio completely switched from a preponderance of IL-6 at 0100 h versus a preponderance of TNF between 0500 h and 0900 h (Fig. 2C).

Molar ratios of serum levels of adrenal hormones

At 4 h after endotoxin injection (2 h after cytokine maximum), the molar ratio of serum cortisol/17OH-progesterone and serum cortisol/DHEA was markedly higher in subjects receiving 0.4 ng endotoxin/kg b.w. as compared with subjects under control conditions (Fig. 3).

This was very similar for the ratio of serum cortisol/DHEAS at the same time point (endotoxin vs control: 0.252 ± 0.200 vs 0.016 ± 0.003 , $P=0.014$). During the following time, these ratios were similar in both experimental groups (Fig. 3). In the dose-response study, 4 h after endotoxin injection, subjects receiving either 0.4 or 0.8 ng endotoxin/kg b.w. demonstrated a significantly elevated molar ratio of serum cortisol/serum DHEA (Fig. 4). Furthermore, high serum levels of IL-6 in relation to TNF were correlated with high serum levels of cortisol in relation to DHEA or 17OH-progesterone (Fig. 5).

Discussion

This study demonstrated an early endotoxin-induced predominance of cortisol in relation to DHEA, DHEAS or 17OH-progesterone 4 h after LPS injection, which

Figure 2 Time course of serum levels of TNF and IL-6 after i.v. endotoxin injection in healthy male volunteers. Serum levels of TNF (A) and IL-6 (B) are depicted in pg/ml. (C) The ratio of serum TNF/serum IL-6. Filled symbols represent data $\pm$ S.E.M. of healthy subjects under control conditions and open symbols demonstrate serum levels of subjects after i.v. injection of 0.4 ng endotoxin per kg b.w. The rectangle in (C) demonstrates the dissociation of the two curves in patients with/without prior endotoxin administration. * $P < 0.05$, ** $P < 0.01$ versus subjects without prior endotoxin administration.

followed a relative increase of serum IL-6 in relation to serum TNF 2 h after LPS injection. A very similar hormone predominance can be observed in chronic inflammatory disease for serum levels of cortisol relative to DHEAS (Straub *et al.* 1998b, Zietz *et al.* 2000) and for serum levels of cortisol relative to DHEA (Straub *et al.* 1998b). In more acute situations such as early untreated rheumatoid arthritis only an increase of cortisol relative to DHEAS but not relative to DHEA was observed (Straub *et al.* 2002). Patients with early untreated rheumatoid arthritis have no positive relation between indices of inflammation and cortisol, but serum DHEAS or DHEA levels are inversely correlated with these inflammatory indices (Cutolo *et al.* 1999, Kanik *et al.* 2000). In acute reactive arthritis, which is a milder form of an inflamma-

tory arthritic disease, no such predominance of cortisol in relation to DHEA, DHEAS or 17OH-progesterone was observed (Straub *et al.* 2002). In acute cholestasis, a similar predominance of cortisol in relation to DHEAS was observed (DHEA was not measured) (Zietz *et al.* 2001a). This indicates that adrenal steroid production is changed into the direction of cortisol relative to adrenal androgen such as DHEA and, particularly, DHEAS depending on the degree and duration of inflammation. Similar hormonal predominance is observed in critically ill patients, and it was mentioned that these changes of relative biochemical pathway predominance may be a factor necessary for survival during chronic severe stress (Parker *et al.* 1985, Drucker & McLaughlin 1986). However, the reasons for this pathway predominance are still

Figure 3 Time course of hormonal ratios after i.v. endotoxin injection in healthy male volunteers. The ratio of serum cortisol/serum 17OH-progesterone (A), and the ratio of serum cortisol/serum DHEA (B) are depicted. Filled symbols represent data $\pm$ S.E.M. of healthy subjects under control conditions and open symbols demonstrate serum levels of subjects after i.v. injection of 0.4 ng endotoxin per kg b.w. The rectangles demonstrate the dissociation of the two curves in patients with/without prior endotoxin administration.

unknown because no adequate methods are yet available to investigate directly human adult adrenocortical cells of these patients *in vivo* or *in vitro*. Thus, only indirect methods can be applied in order to shed light on this obscure phenomenon.

Some key mediators of increased serum levels of cortisol relative to DHEA, DHEAS or 17OH-progesterone may be (a) circulating cytokines such as TNF and IL-6, which exert their specific effects at the level of the adrenocortical cell (Jäättelä *et al.* 1991, Pärth *et al.* 1996, Barney *et al.*

Figure 4 Endotoxin dose-response for the ratio of serum levels of cortisol/DHEA at the time point 0300 h. Underneath the bars, the different injected doses are indicated in ng endotoxin/kg b.w. Filled (open) bars represent means $\pm$ S.E.M. of subjects without (with) prior endotoxin injection.

2000), (b) local immune cells in the adrenal gland, which produce cytokines or act via surface molecules (Wolkersdorfer *et al.* 1999), and (c) neurotransmitters or neuropeptides of innervating nerves of the adrenal glands, whose release may be under control of local cytokines (Ehrhart-Bornstein *et al.* 1998). Since TNF can inhibit the secretion of cortisol from adrenal cells due to an inhibition of the P450c21 or other factors (Jäättelä *et al.* 1991, Barney *et al.* 2000), and IL-6 increases cortisol secretion (Pärth *et al.* 1996, Barney *et al.* 2000), these cytokines may be relevant for early increased secretion of cortisol relative to DHEA, DHEAS or 17OH-progesterone during endotoxaemia or chronic inflammatory diseases. In our patients with endotoxaemia, this effect of TNF and IL-6 may be confirmed by the correlation of the ratio of serum IL-6/TNF and the ratio of serum cortisol/DHEA (DHEAS, or 17OH-progesterone). This indicates that elevated levels of IL-6 in relation to TNF are associated with elevated cortisol relative to DHEA, DHEAS or 17OH-progesterone. A very similar finding has been described recently for serum cortisol relative to serum 17OH-progesterone in early untreated patients with rheumatoid arthritis (Straub *et al.* 2002). Although this is only indirect evidence from a deductive stand-point, the mentioned inductive experiments with fetal human or bovine adrenocortical cells may confirm this view.

Interestingly, with ageing, a very similar pathway predominance appears, with elevated serum levels of cortisol relative to DHEA or DHEAS (Belanger *et al.* 1994, Ferrari *et al.* 2001). During ageing, a similar increase of IL-6 appears which is not paralleled by a similarly intense

Figure 5 Correlation between cytokine ratio and hormone ratio at the time point 0300 h. (A) The interrelation between the ratio of serum IL-6/TNF and the ratio of serum cortisol/DHEA. (B) The correlation between the ratio of serum IL-6/TNF and the ratio of serum cortisol/17OH-progesterone. The linear regression line, the Spearman rank correlation coefficient (R_{Rank}) and its P -value are given. Filled (open) symbols represent subjects without (with) prior endotoxin administration.

increase of TNF (Straub *et al.* 1998a, Zietz *et al.* 2001b). In this situation, one may speculate that very similar effects modulate adrenal cortisol and androgen production and, possibly, locally or systemically produced cytokines are the main players of these hormonal changes. During ageing, increased adipose tissue may be a relevant source of elevated serum levels of IL-6 but not of TNF (Mohamed-Ali *et al.* 1997, Fried *et al.* 1998).

In conclusion, dissociation of cortisol relative to DHEA, DHEAS or 17OH-progesterone appears very early during a systemic inflammatory response which followed an increase of IL-6 relative to TNF. This would suggest a very different role for IL-6 compared with TNF, where IL-6 stimulates and TNF inhibits adrenal steroidogenesis (particularly adrenal androgens). These hormonal changes during an inflammatory response are probably necessary to achieve adequate cortisol levels at the expense of adrenal androgens. Such a relative increase of cortisol is necessary in order to overcome acute severe illness which has been demonstrated in sepsis experiments (Hinshaw *et al.* 1985). However, in chronic inflammatory diseases, maintenance of this pathway predominance and a generally lower production of adrenal steroids may be deleterious. In these diseases, together with glucocorticoids, DHEA may be a relevant anti-inflammatory agent.

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