CLINICAL STUDY

During a corticotropin-releasing hormone test in healthy subjects, administration of a beta-adrenergic antagonist induced secretion of cortisol and dehydroepiandrosterone sulfate and inhibited secretion of ACTH

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Abstract

Objective: In chronic inflammatory diseases, serum levels of dehydroepiandrosterone (DHEA) sulfate (DHEAS) are low. Interestingly, several non-inflammatory diseases display similarly low levels of DHEAS which points to other inhibitory factors such as an activated sympathetic nervous system (SNS) (e.g. in patients with heart failure, fibromyalgia, or cancer cachexia). We aimed to identify the influence of the SNS tone on stimulated adrenal steroid secretion in 16 male and 12 female healthy subjects.

Methods: One group were given oral propranolol 2 h before a corticotropin-releasing hormone (CRH) test, and levels of adrenocorticotropin (ACTH), cortisol, 17-hydroxyprogesterone (17OHP), androstenedione, DHEA, and DHEAS were measured.

Results: Propranolol treatment decreased heart rate (by 20%), diastolic blood pressure (by 20%), and plasma ACTH, and increased serum cortisol, serum DHEAS, and the molar ratio of cortisol/17OHP, cortisol/DHEA, and DHEAS/DHEA similarly in female and male subjects.

Conclusions: A β -adrenergic influence seems to decrease CRH-stimulated cortisol in relation to ACTH and 17OHP, and decreases DHEAS in relation to DHEA. Although other workers have found β -adrenergic stimulation of steroid secretion in cultured adrenocortical cells, the overall systemic influence of the SNS via β -adrenoceptors seems to inhibit adrenal steroids under unstimulated and stimulated conditions. Sympathetic hyperactivity may be a common denominator for low levels of DHEAS in inflammatory and non-inflammatory diseases.

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Introduction

Chronic inflammatory diseases such as rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, and others are accompanied by low levels of dehydroepiandrosterone (DHEA) and its sulfated derivative DHEAS (1–8). The reason for the unexpected decrease in serum levels of adrenal androgens during chronic inflammation is not yet known. It has been proposed that proinflammatory cytokines such as tumor necrosis factor (TNF) and intra-adrenal immune cells may change the microenvironment leading to lower adrenal and gonadal hormone secretion in chronic inflammatory diseases but, up to now, no formal proof has been presented (9).

Interestingly, non-inflammatory diseases such as fibromyalgia, heart failure, cardiovascular disease, and cancer similarly display low levels of DHEAS (10–16). Under these non-inflammatory conditions,

cytokines or intra-adrenal immune cells probably will not play a major role in changes in adrenal DHEAS secretion. Thus, other factors which are similarly altered in the same direction in inflammatory and non-inflammatory disease may be more relevant. The common denominator for this phenomenon in inflammatory and non-inflammatory diseases may be a hypersympathetic nervous tone which has been demonstrated several times in the mentioned diseases with low DHEAS levels (e.g. 17–22).

Since the sympathetic nervous system via β -adrenoceptors has a strong influence on adrenal hormone secretion (23), we investigated the influence of the non-selective β -adrenergic antagonist propranolol (without intrinsic sympathetic activity) on corticotropin-releasing hormone (CRH)-stimulated secretion of adrenal hormones. In contrast to two earlier studies with propranolol and CRH injection (24, 25) or propranolol and hypoglycemia (e.g. 24, 26, 27), this study

extended the earlier view by consideration of molar hormone ratios such as cortisol/adrenocorticotropin (ACTH), cortisol/17-hydroxyprogesterone (17OHP), cortisol/DHEA, and DHEAS/DHEA. Furthermore, responses were separately calculated and depicted for female and male subjects.

Subjects and methods

Subjects

A total of 28 healthy subjects, 12 women (aged 25 years, range 21–33 years) and 16 men (aged 26 years, range 23–36 years), were included in this study. The subjects were checked by a 33-item questionnaire (28), which revealed that these subjects had had no severe diseases in the past and no present or current symptoms of diseases. They were not on medication, had not had prior vaccination, did not smoke or drink alcohol in excess, and their family and surgical histories were unremarkable. The questionnaire was adapted from the SENIEUR protocol (29). The women in this study were in the early to mid follicular phase of the menstrual cycle in order to be comparable to their male counterparts, and they were not taking oral contraceptives. The study was approved by the Ethics Committee of the University of Regensburg and the subjects were extensively informed about the study protocol and gave written consent. Groups with or without prior propranolol administration (see below) were not different in age (nine males without propranolol: 26 ± 1 , seven males with prior propranolol: 26 ± 1 , five females without prior propranolol: 27 ± 2 , seven females with prior propranolol: 24 ± 1 years).

Test procedure

At 0700 h, the study subjects had a light breakfast and they were allowed to drink mineral water during the morning. Then, they were admitted to the ambulatory research unit at 1200 h. They were instructed to remain in a supine position for the entire duration of the test. They received an indwelling catheter in an antecubital vein at 1215 h (for i.v. injection of human (h) CRH, and for withdrawing blood samples) and blood pressure was measured at the contralateral arm. They were connected to an ECG monitor, and heart rate, beat-to-beat intervals, and blood pressure were recorded. In the propranolol-treated group, 10 mg propranolol (Dociton, Astra-Zeneca, Plankstadt, Germany) were given orally. Target heart rate and diastolic blood pressure were adjusted to levels 20% lower than the initial value by additional propranolol tablets (10 mg) if necessary (this occurred in only two subjects, one hour after the first administration). The first blood sample was drawn at time -10 min (1350 h). One hundred micrograms hCRH (Ferring Arzneimittel, Kiel, Germany) were administered i.v. as a bolus

10 min later at time 0 (1400 h). Blood samples were taken at the following experimental times: -10 , $+15$, $+30$, $+45$, $+90$ and $+120$ min. The samples were immediately centrifuged and plasma and serum were stored in adequate aliquots at -80°C . Heart rate, diastolic and systolic blood pressure, and heart rate variability during 6 deep breaths per minute (respiratory sinus arrhythmia test (30)) were recorded at the following experimental times: -10 , $+5$, $+30$, $+45$, $+90$ and $+120$ min.

All tests were started at 1400 h when the tonus of the hypothalamic–pituitary–adrenal (HPA) axis and of the hypothalamus–autonomic nervous system (HANS) axis were in an intermediate stage, which allowed for the investigation of either upregulation or downregulation of responses. The test ended at 1600 h, the catheter and ECG electrodes were removed, and the subjects were observed for another 30 min.

Laboratory methods

Several adrenal hormones were considered in order to detect major adrenal pathways of steroidogenesis. We used radioimmunoassays for the quantitative determination of serum levels of cortisol (Coulter Immunotech, Marseilles, France; detection limit: 10 nmol/l). Serum levels of 17OHP (IBL, Hamburg, Germany; detection limit: 0.3 nmol/l), androstenedione (ASD) (IBL; detection limit: 0.13 nmol/l), DHEAS (IBL; detection limit: 130 nmol/l), DHEA (Diagnostic Systems Laboratory, Webster, TX, USA; detection limit: 0.13 nmol/l), and plasma levels of ACTH (Sangui Bio-Tech, Inc., CA, USA, via IBL; detection limit: 0.1 pmol/l) were measured by enzyme immunoassays. In all tests, interassay and intra-assay variability were lower than 10%.

Calculation of hormone/hormone ratios

In order to demonstrate the shift from one serum hormone to another, the molar ratio of these hormones was calculated (given without units) (31, 32). This procedure detects a hormonal shift through one or more adrenal enzyme steps which can demonstrate a preponderance of an adrenal pathway: cortisol/17OHP for the pathway through P450c21 and P450c11 into the direction of cortisol, ASD/17OH-progesterone for the 17,20lyase (2nd reaction of the P450c17) into the direction of ASD, DHEA/ASD for the 3β -hydroxysteroid dehydrogenase into the direction of DHEA, and DHEAS/DHEA for the combined reaction of the DHEA sulfotransferase and the DHEAS sulfatase into the direction of DHEAS. Finally, the molar ratio of serum cortisol/serum DHEA indicates a shift into the direction of cortisol in relation to adrenal androgens.

Statistical analysis

Responses of the different subgroups in relation to the observation time were compared by multivariate analysis (general linear model, SPSS/PC for Windows, v.10.0.5, SPSS, Inc., Chicago, IL, USA) after having tested homogeneity of variances. Values are expressed as means $\pm$ S.E.M. and the significance level was $P < 0.05$.

Results

Inhibition of the sympathetic nervous system

In order to control inhibition of the sympathetic nervous system, heart rate, diastolic blood pressure, and heart rate variability during the respiratory sinus arrhythmia test were recorded. Figure 1 demonstrates

a 20% reduction of heart rate and diastolic blood pressure and a significant reduction in heart rate variability in the respiratory sinus arrhythmia test in subjects receiving propranolol. These data indicate a clear inhibition of the sympathetic nervous system by propranolol before and during the hCRH test, irrespective of gender (Fig. 1).

Plasma ACTH and serum cortisol in relation to each other

Figure 2 demonstrates that plasma levels of ACTH were significantly lower in both male and female subjects in the group with prior propranolol administration. As compared with control subjects, in subjects with propranolol, the increase in ACTH was delayed and the ACTH peak appeared at 45 min irrespective of gender

Figure 1 Time course of autonomic nervous function parameters before and during a hCRH test with or without prior propranolol administration in women and men. The figure depicts heart rate (A, B), diastolic blood pressure (C, D), and heart rate variability of the respiratory sinus arrhythmia test (E, F). The unit of the respiratory sinus arrhythmia test is the age-corrected percentile localization, as described earlier (30). One hundred micrograms hCRH were injected at 1400 h and parameters were recorded at -10, +5, +30, +45, +90 and +120 min. Solid symbols represent data without prior propranolol administration and open symbols represent data with prior propranolol administration. Data are given as means $\pm$ S.E.M. The P value gives the significance for the comparison of the different subgroups in relation to the observation time compared by multivariate analysis (general linear model): Co, control subjects; Doc, propranolol-treated subjects.

Figure 2 Time course of ACTH, cortisol, and the hormone ratio before and during a hCRH test with (○) or without (●) prior propranolol administration in women and men. The figure depicts plasma ACTH (A, B), serum cortisol (C, D), and the ratio of serum cortisol/plasma ACTH (E, F). The molar ratio has no units. One hundred micrograms hCRH were injected at 1400 h and parameters were recorded at -10, +15, +30, +45, +90 and +120 min. Explanations for symbols, data presentation, *P* value, and abbreviations are given in the legend to Fig. 1.

(Fig. 2A,B). In contrast, serum cortisol was significantly elevated in propranolol-treated women and men as compared with control subjects (Fig. 2C,D). Serum cortisol demonstrated the typical hCRH-dependent increase with a peak at 45 min irrespective of gender and treatment (Fig. 2C,D). This led to a significantly elevated molar cortisol to ACTH ratio in the propranolol group compared with control subjects which was independent of gender (Fig. 2E,F).

The second step of P450c17 and 3 β -hydroxysteroid dehydrogenase

Propranolol treatment did not change serum levels of ASD (Fig. 3A,B), the ratio of serum DHEA/serum ASD, or serum ASD/serum 17OHP irrespective of gender (data not shown).

The adrenal steroid pathway via P450c21 and P450c11

Women with prior propranolol administration demonstrated significantly lower serum levels of 17OHP as compared with the control group, which was not obvious in male subjects (Fig. 3C,D). Concerning the ratio of serum cortisol/serum 17OHP, women receiving propranolol displayed a significantly elevated ratio as compared with the control group (Fig. 3E). Although smaller in size, this effect was also observed in male subjects with prior propranolol administration (Fig. 3F).

The DHEA sulfotransferase and the DHEAS sulfatase reaction

Administration of propranolol to women and men led to a significant increase in serum DHEAS (Fig. 4A,B), which was not observed for serum DHEA (Fig. 4C,D). In the propranolol group, the molar ratio of serum

Figure 3 Time course of androstenedione, 17-hydroxyprogesterone (17OHP) and the ratio of serum cortisol/serum 17OHP before and during a hCRH test with (○) or without (●) prior propranolol administration in women and men. The figure depicts serum androstenedione (A, B), 17OHP (C, D), and the ratio of serum cortisol/serum 17OHP (E, F). The molar ratio has no units. Procedure and explanations for symbols, data presentation, *P* value, and abbreviations are given in the legend to Fig. 1.

DHEAS/serum DHEA was significantly elevated as compared with control subjects (Fig. 4E,F). This was independent of gender and appeared to be more significant under unstimulated than under stimulated conditions (i.e. at baseline as compared with conditions after CRH injection).

Two main adrenal steroid pathways versus each other: serum cortisol versus serum DHEA

Propranolol treatment increased the molar ratio of serum cortisol/serum DHEA under unstimulated and stimulated conditions in women and men (Fig. 5A,B). The propranolol-induced increase was more marked in female as compared with male subjects (Fig. 5A,B).

Discussion

To our knowledge, no other studies are available which investigated adrenal steroid pathways in a similar way with and without propranolol pre-treatment and concomitant CRH stimulation. One study has shown a methoxamine (α 1-adrenergic agonist)-induced increase of CRH-stimulated ACTH and subsequent serum cortisol increase which are compatible with our results (25). In another experiment, the unspecific α 1,2-adrenergic antagonist, phentolamine, had no influence on CRH-stimulated ACTH and cortisol levels (24). Several studies induced hypoglycemia in order to activate the HPA axis and administered β -adrenergic and α -adrenergic agonists and antagonists: four independent studies noted a propranolol-induced increase in plasma ACTH and/or serum cortisol during hypoglycemia (24, 26, 27, 33). However, these groups did not

Figure 4 Time course of DHEAS, DHEA, and the ratio of DHEAS/DHEA before and during a hCRH test with (○) or without (●) prior propranolol administration in women and men. The figure depicts serum DHEAS (A, B), serum DHEA (C, D), and the ratio of serum DHEAS/serum DHEA (E, F). The molar ratio has no units. Procedure and explanations for symbols, data presentation, *P* value, and abbreviations are given in the legend to Fig. 1.

study adrenal hormone cascades. Two other studies with β -adrenergic antagonists other than propranolol did not demonstrate an increase in either plasma ACTH or serum cortisol (34, 35). Similar to studies with CRH and α -adrenergic antagonists, studies with

hypoglycemia-induced activation of the HPA axis and administration of α -adrenergic antagonists did not change either plasma ACTH or serum cortisol levels (36–38). One very interesting study with i.v. ACTH and prior propranolol administration revealed an

Figure 5 Time course of the ratio of serum cortisol/serum DHEA before and during a hCRH test with (○) or without (●) prior propranolol administration in women and men. The molar ratio has no units. Procedure and explanations for symbols, data presentation, *P* value, and abbreviations are given in the legend to Fig. 1.

increase in serum cortisol (39). Taken together, prior propranolol administration increased serum cortisol independent of the stimulus, whether hypoglycemia, i.v. ACTH, or i.v. CRH. This indicates that under such systemic conditions a sympathetic nervous system (SNS) tone via β -adrenoceptors decreases serum cortisol. Interestingly, administration of epinephrine to healthy subjects over one hour also reduced serum cortisol levels, whereas short-term administration over 20 min was ineffective (R H Straub, unpublished observation within another study, (40)). Our study adds the information that under such systemic conditions an SNS tone via β -adrenoceptors also decreases the ratio of cortisol/ACTH, cortisol/17OHP, cortisol/DHEA, and DHEAS relative to DHEA. To our knowledge, no *in vitro* studies are available which tested the direct influence of β - or α -adrenergic agonists or antagonists on adrenal DHEA sulfotransferase or DHEAS sulfatase which may have explained the latter phenomenon. One has to mention that none of these effects may be direct on the adrenocortical level but may be more systemic since β -adrenergic receptors were not found on normal human adrenocortical cells but only on tumor cells (41–43).

At this point, we have to mention that we conducted our study in four different groups of female and male healthy subjects with and without propranolol. It is a disadvantage of our study that we were not able to recruit the same group a second time in a cross-over design. This may have influenced the general serum levels of steroid hormones at baseline and during the CRH test between the groups. However, we believe that this fact should not have influenced the results to a large extent since the mean age was very similar between the groups and the variation at baseline and during the test was relatively small as indicated by the S.E.M. Furthermore, the effects of propranolol administration are similar in the two series with either female or male subjects.

These data and results of the studies mentioned seem to be at odds when compared with several studies with β -adrenergic agonists and human and bovine adrenocortical cells in culture. It has been repeatedly demonstrated that stimulation of the β -adrenoceptor on adrenocortical cells increases cortisol secretion (44–49). Furthermore, the group of Bornstein *et al.* even demonstrated up-regulation of important steroidogenic enzymes such as P450scc, P450c17, P450c21, and P450c11 (47). In another study, they demonstrated that co-culture of bovine adrenocortical cells and chromaffin cells increases cortisol secretion up to 10-fold (49). At the moment, these contrasting findings cannot be explained by our present data and the literature available. One may speculate that additional co-factors must be present in the local microenvironment which lead to these results. Nevertheless, from the perspective of the entire body a β -adrenergically induced decrease in serum levels of adrenal hormones seems to be obvious.

Above, we mentioned the strong decrease in serum DHEAS in chronic inflammatory and non-inflammatory diseases. In earlier literature on chronic inflammatory diseases, this phenomenon was ascribed to proinflammatory changes of the adrenal microenvironment but no formal proof has ever been presented. In the case of non-inflammatory diseases, proinflammatory immune-mediated changes should not play a role, however, DHEAS serum levels are also low under these conditions. We now propose that SNS hyperactivity may be the common denominator for low levels of DHEAS in inflammatory and non-inflammatory diseases. In both disease groups, SNS hyperactivity has been described (see for example 17–22), and, in this study, systemic inhibition of β -adrenergic pathways by propranolol on multiple systemic levels leads to an increase in serum DHEAS and to an increased serum DHEAS/serum DHEA ratio.

In conclusion, a β -adrenergic influence seems to decrease CRH-stimulated cortisol in relation to ACTH and 17OHP, and leads to a decrease in DHEAS in relation to DHEA. Although others found β -adrenergic stimulation of steroid secretion of cultured adrenocortical cells, the overall systemic influence of the SNS via β -adrenoceptors seems to inhibit adrenal steroids under unstimulated and stimulated conditions. We propose that sympathetic hyperactivity may be the common denominator for low levels of DHEAS in inflammatory and non-inflammatory diseases.

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