

Original article

Lack of dehydroepiandrosterone in type I and II hereditary angioedema and role of danazol in steroid hormone conversion

Background: Hereditary angioedema (HAE) is successfully treated with danazol, a therapeutic steroid compound. To investigate hormones of the hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-gonadal (HPG) axis in patients with HAE with and without danazol.

Methods: We included 16 patients with type I HAE, nine patients with type II HAE, and 16 healthy subjects. Serum levels of adrenocorticotrophic hormone (ACTH), cortisol, androstenedione, dehydroepiandrosterone (DHEA), free testosterone, and 17 β -oestradiol were measured.

Results: Serum levels of ACTH were markedly decreased in patients with type II HAE compared to the other groups ($P < 0.001$). Serum cortisol was similar between groups but danazol treatment decreased cortisol levels, particularly in women ($P = 0.019$). Serum levels of DHEA were significantly decreased in all patients with type I and II HAE compared to controls ($P < 0.05$), which was only partly dependent on prior danazol therapy as patients without danazol had also decreased serum levels of DHEA ($P < 0.05$). Furthermore, free testosterone serum levels were markedly increased in patients under danazol ($P < 0.005$) and the ratio of 17 β -oestradiol/free testosterone was significantly decreased in these patients ($P < 0.005$).

Conclusions: This study demonstrated decreased DHEA in patients with type I and II HAE independent of danazol therapy, which was particularly evident in women. It also demonstrates that danazol induced a marked up-regulation of free testosterone in relation to precursors and downstream 17 β -oestradiol. In HAE, there seems to be a primary lack of the adrenal androgen DHEA.

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Key words: adrenocorticotrophic hormone; cortisol; danazol; dehydroepiandrosterone; hereditary angioedema; testosterone.

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Hereditary angioedema (HAE) with defective inhibitor of the C1 esterase (C1-INH) function predisposes to episodes of self-limited, increased vascular permeability that is restricted to three specific sites: subcutaneous space, gut and upper airways (1–3). Two types of HAE have been described (4): type I HAE, which occurs in approximately 80% of patients, is caused by decreased production of C1-INH. Patients with type II HAE have normal or elevated quantities of functionally impaired C1-INH (1). Recently, an oestrogen-dependent type with functionally normal C1-INH has been described in women but the underlying defect is not yet known (5). Recurrent angioedema are also seen in the acquired defect of C1-INH that is because of autoantibodies against this

protein or an associated disease causing accelerated catabolism of C1-INH (6).

As the seminal work of Gelfand et al. (7) in the 1970s, danazol, a 17 α -acetylated androgen, is used in patients with HAE as long-term prophylactic agent. These androgens can prevent attacks in type I and II HAE patients. In case reports, it has been demonstrated that hypoandrogenism can be linked to HAE (8). It has been shown that androgens such as testosterone and dehydroepiandrosterone (DHEA) can upregulate C1-INH from monocytic and hepatoma cell lines (9). Interestingly, no studies exist which have systematically investigated hormones of the hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-gonadal (HPG) axis in a larger group of patients with HAE. It may be possible that pre-existing alterations of these hormonal axes exist independent of androgen therapy. The aim of this study was to investigate hormones of the HPA and HPG axis in type I and II HAE under additional consideration of danazol therapy, which is known to alter steroid cascades.

Abbreviations: ACTH, adrenocorticotrophic hormone; ASD, androstenedione; C, complement; C1-INH, inhibitor of the C1 esterase; DHEA, dehydroepiandrosterone; DHEAS, DHEA sulphate; HAE, hereditary angioedema; HPA axis, hypothalamic-pituitary-adrenal axis; HPG axis, hypothalamic-pituitary-gonadal axis; IL, interleukin; SHBG, sex hormone binding globulin.

Materials and methods

Patients and controls

We included 16 patients with type I HAE, nine patients with type II HAE and 16 healthy subjects of the same ethnic group (white patients). These patients were collected in St Anne Faculty Hospital, Brno, The Czech Republic and ethical approval was given by the Ethics Committee of the University of Brno. The characteristics of patients and controls are given in Table 1. Blood samples were drawn between 09:00 and 11:00 hours from all patients. Typically, no overt signs of inflammation are present in HAE patients, which is in our study best indicated by normal serum levels of interleukin (IL)-6 (Table 1) and by normal acute phase proteins (data not given). Hereditary angioedema patients demonstrated the typical abnormalities of the classical complement pathway (Table 1). In another investigation, we compared healthy subjects with the same HAE patients with and without danazol treatment. The characteristics of the groups are given in Table 2.

Table 1. Basic characteristics of subjects under study

	Healthy subjects	Type I HAE	Type II HAE
Number	16	16	9
Age (year)	37.9 ± 2.8 [20–58]	31.4 ± 4.2 [8–60]	38.6 ± 4.7 [18–57]
Women/men	8 / 8 (50/50)	8/8 (50/50)	5 / 4 (44/56)
C1-INH(mg/l)	287 ± 9	69 ± 7**	606 ± 80***
C2 (mg/l)	16.3 ± 0.7	7.9 ± 0.8**	8.1 ± 1.6**
C4 (g/l)	0.23 ± 0.01	0.06 ± 0.01**	0.08 ± 0.02**
CH50 (%)	91.9 ± 2.7	53.2 ± 7.0**	54.1 ± 7.7**
IL-6 (pg/ml)	4.3 ± 0.3	4.8 ± 1.0	4.4 ± 0.4
Current medication†			
Glucocorticoids, n (%)	n.a.	0 (0)	0 (0)
Danazol, n (%)	n.a.	4 (25)	6 (67)*
Tranexamic acid, n (%)	n.a.	6 (38)	3 (33)

Data are given as means ± SEM, percentages in parentheses, and ranges in brackets.

HAE, hereditary angioedema; C, complement; C1-INH, inhibitor of the C1 esterase; IL-6, interleukin-6; n.a., not applicable.

* $P = 0.046$ vs patients with C1-INH deficiency; ** $P < 0.001$ vs healthy subjects;

*** $P < 0.001$ vs healthy subjects and patients with C1-INH deficiency.

†The patients did not receive any other medication.

Table 2. Characteristics of the same HAE patients as in Table 1 with and without danazol administration

	Patients without danazol	Patients with danazol
Number	15	10
Age (year)	27.4 ± 3.2 [10–57]	43.8 ± 5.1 [8–60]*
Women/men	6/11 (40/60)	7/10 (70/30)
HAE type 1 and 2	12/3 (80/20)	4/6 (40/60)*
C2 (mg/l)	6.8 ± 0.9	9.7 ± 1.1*
C4 (g/l)	0.04 ± 0.01	0.09 ± 0.01**
CH50 (%)	53.8 ± 6.8	54.8 ± 8.1

Data are given as means ± SEM, percentages in parentheses, and ranges in brackets.

HAE, hereditary angioedema; C, complement; n.a., not applicable.

* $P < 0.05$; ** $P < 0.005$ vs patients without danazol.

Analysis of complement factors

Inhibitor of C1 esterase and complement 4 (C4) were measured by nephelometry (Dade Behring BN II, Marburg, Germany) using the commercially available reagents. Complement 2 was measured by radial immunodiffusion (The Binding Site, Birmingham, UK). CH50 was measured by using the standard haemolytic assay with sheep red blood cells. We also confirmed the C1-INH deficiency using the functional enzyme-linked immunosorbent assay (Quidel, San Diego, CA, USA).

Analysis of hormones

Several adrenal and gonadal hormones were considered to detect major pathways of steroidogenesis. Figure 1 demonstrates important steroid cascades. We used a radioimmunoassay for the quantitative determination of serum cortisol (Coulter Immunotech, Marseilles, France; detection limit: 10 nmol/l). Serum levels of androstenedione (IBL, Hamburg, Germany; detection limit: 0.15 nmol/l), DHEA (Diagnostic Systems Laboratory, Webster, Texas; detection limit: 0.13 nmol/l), DHEA sulphate (DHEAS, IBL, Hamburg, Germany; detection limit: 130 nmol/l), free testosterone (IBL, Hamburg, Germany; detection limit: 5.2 pmol/l), and 17 β -oestradiol (IBL, Hamburg, Germany; detection limit: 58.7 pmol/l) were measured by means of immunometric enzyme immunoassays. Serum levels of IL-6 (high sensitivity Quantikine, R&D Systems, Minneapolis, MN, USA; detection limit: 0.2 pg/ml) were measured using the same technique.

Using a sensitive enzyme immunoassay for adrenocorticotrophic hormone (ACTH, Sangui BioTech, Inc., CA, USA, via IBL, Hamburg, Germany; detection limit: 0.1 pmol/l), we were able to demonstrate a highly significant interrelation between ACTH measured in serum and ACTH assayed in plasma with the following regression equation: ACTH (plasma) = 7.2508 + 1.8707 × ACTH (serum), $R = 0.646$, $P < 10^{-6}$, $n = 112$ (10). In this work, ACTH levels are given as serum levels. For all assays, intraassay and interassay coefficients of variation were below 10%.

Statistical analysis

To compare medians in two different groups the Mann–Whitney test was used (SPSS/PC for Windows, V.10.0.5, SPSS, Inc., Chicago, IL, USA). The P -values of < 0.05 were considered to be significant and means are always given with ± SEM.

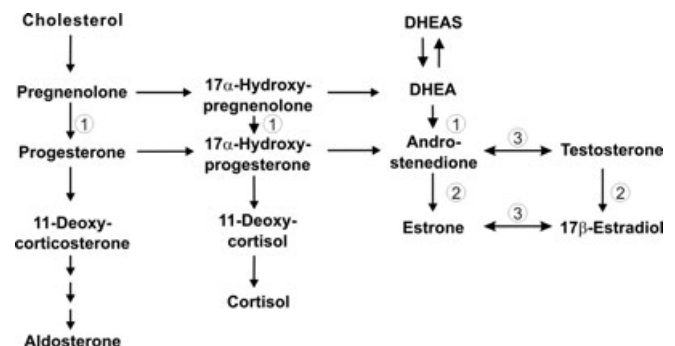


Figure 1. Adrenal and gonadal pathways of steroidogenesis. Enzymes: 1, 3 β -hydroxysteroid dehydrogenase; 2, aromatase; 3, 17 β -hydroxysteroid dehydrogenase. DHEA, dehydroepiandrosterone; DHEAS, DHEA sulphate.

Results

Serum levels of ACTH and cortisol

Patients with type II HAE had significantly decreased levels of serum ACTH in comparison to patients with type I HAE and healthy subjects independent of gender (Fig. 2A). Danazol treatment did not change serum levels of ACTH (Fig. 2B). In contrast to findings with ACTH, serum cortisol levels were similar in all groups (Fig. 2C). However, female patients with prior danazol treatment demonstrated lower serum levels of cortisol in comparison with healthy women and women without danazol (Fig. 2D).

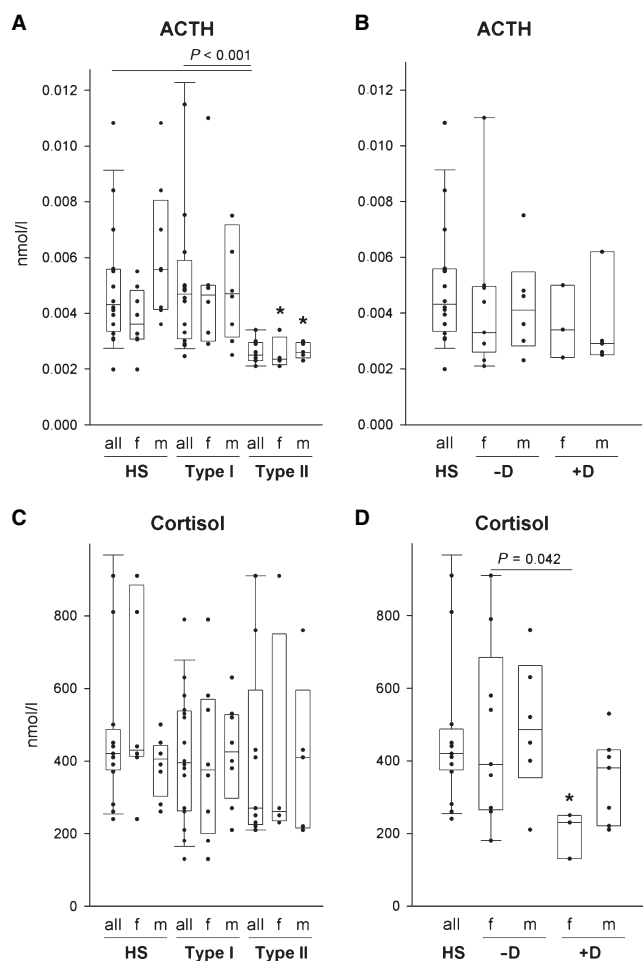


Figure 2. Adrenocorticotrophic hormone (ACTH) and cortisol serum levels in patients with hereditary angioedema. (A) and (C) demonstrate serum levels in healthy subjects compared to patients with type I HAE and type II HAE. (B) and (D) demonstrate serum levels in relation to danazol treatment. *P*-values for comparisons are given on horizontal lines or as symbols: **P* < 0.05 vs the respective sex-matched healthy control given in panel A or vs healthy subjects in panel D. -D, without danazol; +D, with danazol; f, women; HS, healthy subjects; m, men.

Serum levels of DHEAS, DHEA and androstenedione

To study adrenal androgen serum levels, we focussed on the major adrenal hormones DHEAS, DHEA and androstenedione. Serum levels of DHEAS were not different among investigated groups (healthy subjects: 4353 ± 514 , type I HAE: 4008 ± 565 , type II HAE: 4281 ± 891 nmol/l), and danazol treatment did not change DHEAS serum levels (healthy subjects: 4353 ± 514 , without danazol: 3889 ± 566 , with danazol: 4432 ± 849 nmol/l). These findings were independent of gender (data not shown).

However, serum levels of DHEA were significantly lower in all patients of both types of HAE compared with healthy subjects (Fig. 3A). For women, this was more obvious than for men (type II error in men) (Fig. 3A). Furthermore, all patients with or without danazol demonstrated lower serum levels of DHEA in comparison with healthy subjects (Fig. 3B). Again, this was particularly evident for women with or without danazol (Fig. 3B). Serum levels of androstenedione were not different between healthy subjects and the different types of HAE (healthy subjects: 5.8 ± 0.7 , type I HAE: 4.8 ± 0.7 , type II HAE: 5.7 ± 1.5 nmol/l), and danazol treatment did not change androstenedione serum levels (healthy subjects: 5.8 ± 0.7 , without danazol: 4.7 ± 0.9 , with danazol: 5.5 ± 1.2 nmol/l). These results were independent of gender (data not shown). Treatment with tranexamic acid did not influence serum levels of DHEA, DHEAS and androstenedione (data not shown).

To demonstrate hormone conversion via the 3β -hydroxysteroid dehydrogenase (DHEA to androstenedione, enzyme 1 in Fig. 1), the ratio of serum DHEA/serum androstenedione was calculated. This particular ratio was significantly lower in all patients with type II HAE compared with healthy subjects (Fig. 3C). In addition, this ratio was lower in female patients with type I and II HAE compared with healthy women (Fig. 3C). Danazol treatment was related to a decreased ratio of serum DHEA/serum androstenedione in all patients, which was particularly evident in female patients (Fig. 3D). This particular ratio was also lower in female patients without danazol treatment (Fig. 3D).

Serum levels of free testosterone and 17β -oestradiol in relation to danazol treatment

To study the effects of danazol on steroid hormones further, serum levels of free testosterone and 17β -oestradiol were investigated. In patients with prior danazol therapy, serum levels of free testosterone were increased, which was particularly evident in women (type II error in men?) (Fig. 4A). Similarly, the ratio of free testosterone/DHEA was also increased in patients treated with danazol irrespective of gender (Fig. 4B). This indicates

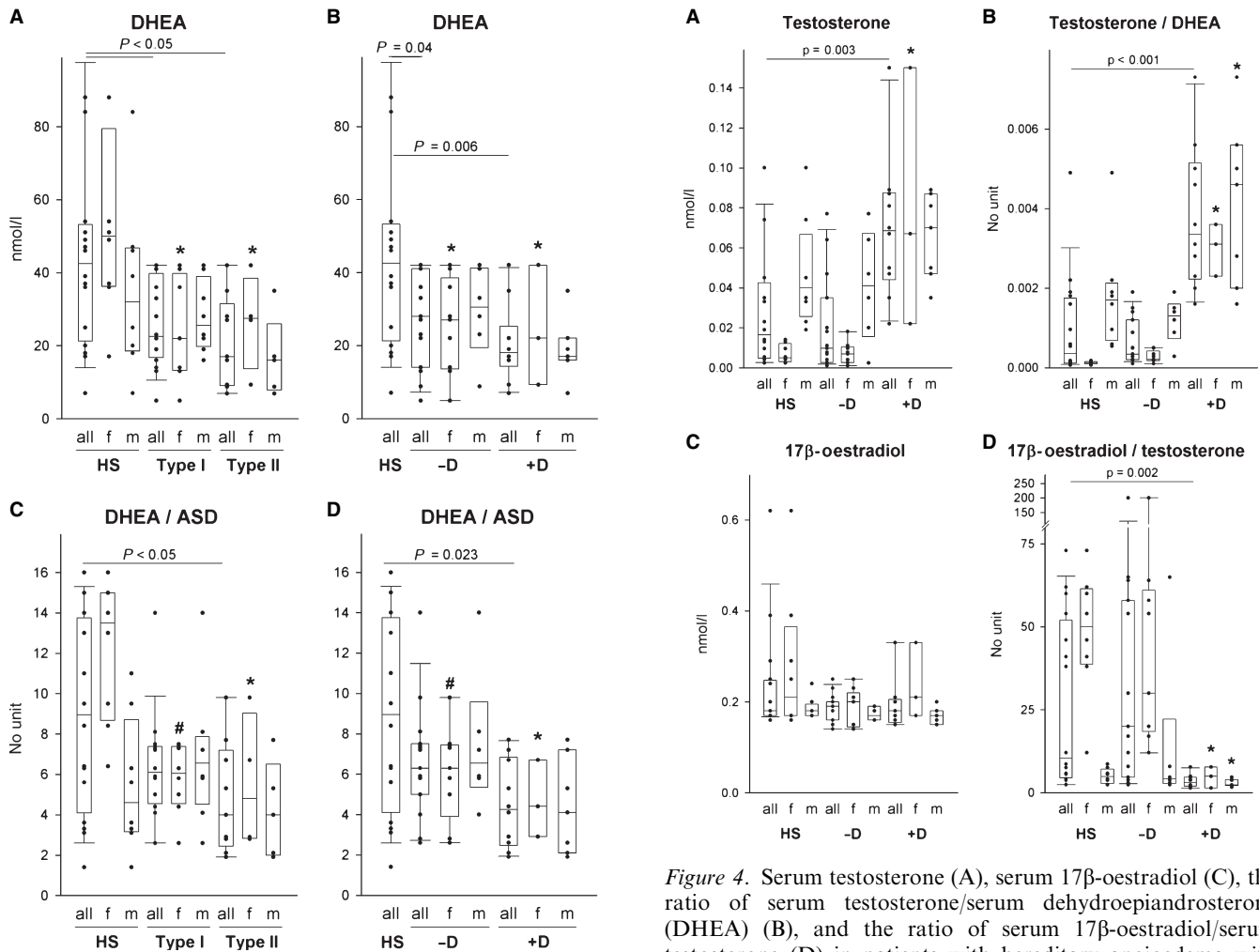


Figure 3. Serum dehydroepiandrosterone (DHEA) and the ratio of serum DHEA/androstenedione (ASD) in patients with hereditary angioedema. (A) and (C) demonstrate values in healthy subjects compared to patients with type I HAE and type II HAE. (B) and (D) demonstrate values in comparison with danazol treatment. *P*-values for comparisons are given on horizontal lines or as symbols: **P* < 0.05 and #*P* < 0.005 vs the respective sex-matched healthy control given in panel A vs healthy subjects in panel B and D, respectively. -D, without danazol; +D, with danazol; f, women; HS, healthy subjects; m, men.

an increased conversion of DHEA to testosterone via the 3 β -hydroxysteroid dehydrogenase and the 17 β -hydroxysteroid dehydrogenase, respectively (enzymes 1 and 3 in Fig. 1).

Serum levels of 17 β -oestradiol were not different among groups irrespective of gender (Fig. 4C). To demonstrate hormone conversion via the aromatase (testosterone to 17 β -oestradiol, enzyme 2 in Fig. 1), the ratio of serum 17 β -oestradiol/serum free testosterone was calculated. This particular ratio was significantly lower in patients treated with danazol irrespective of gender (Fig. 4D).

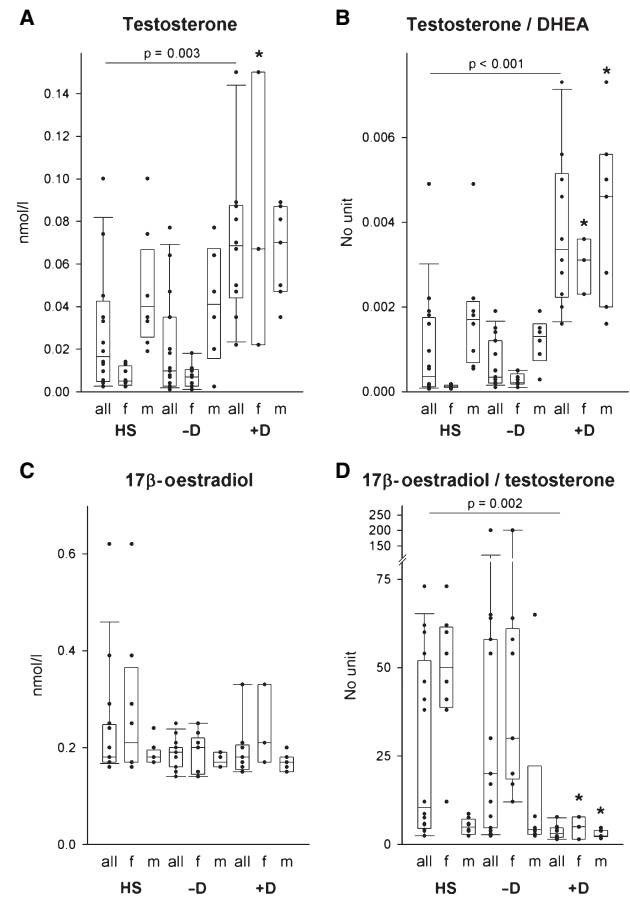


Figure 4. Serum testosterone (A), serum 17 β -oestradiol (C), the ratio of serum testosterone/serum dehydroepiandrosterone (DHEA) (B), and the ratio of serum 17 β -oestradiol/serum testosterone (D) in patients with hereditary angioedema with and without danazol. *P*-values for comparisons are given on horizontal lines or as symbols: **P* < 0.05 vs the respective sex-matched healthy controls. -D, without danazol; +D, with danazol; f, women; HS, healthy subjects; m, men.

Discussion

This study demonstrates decreased serum levels of ACTH in type II HAE compared with type I HAE and healthy subjects. This finding is largely independent from danazol treatment. The lack of ACTH is not linked to alterations in cortisol secretion as cortisol levels were similar between groups. This indicates that cortisol secretion is compensated under these different ACTH conditions in type II HAE. Adrenocorticotrophic hormone, β -endorphin and melanocyte stimulating hormone are encoded by the proopiomelanocortin gene. In this respect, it is interesting that others have demonstrated increased β -endorphin levels in patients with HAE irrespective of the type of HAE (11). Furthermore, this group also demonstrated that β -endorphin secretion from peripheral blood mononuclear cells was increased whereas ACTH was not changed (12). When both ACTH and β -endorphin are encoded by the proopiomelanocortin gene, one might

expect also increased levels of ACTH in HAE. One may speculate that disease-related mechanisms selectively increase β -endorphin secretion whereas ACTH secretion may be suppressed at least in type II HAE. Our extensive search in several data bases did not reveal a genetic or a functional link between the C1-INH(chromosome 11q11-q13.1) and proopiomelanocortin (chromosome 2p23.2), the proprotein convertases (PC) 1 and 2 (PC1: 20p11.2 and PC2: 5q15-q21), or cofactors of PC1 and PC2 such as the secretory granule neuroendocrine protein 1 (SGNP1, 15q11-q15). Currently, this finding remains largely unexplained, and future studies have to address whether such a dissociation of β -endorphin and ACTH really exists in HAE.

A further interesting finding in this study was decreased serum levels of DHEA in HAE compared to controls. It is important that this phenomenon was largely independent of danazol treatment, although danazol treatment seems to amplify this effect. Low androgen levels were particularly evident in female patients, and it was not significant in male patients probably because of a statistical type 2 error. As ACTH is the main stimulus of androgen secretion by inducing the adrenal and gonadal P450c17 gene (13), its loss in type II HAE might lead to a concomitant loss of DHEA. It is unclear why this loss also appears in ACTH-normal type I HAE. Whether or not decreased DHEA secretion depends on the inherited defect of the C1-INH gene remains unknown but the lack of this hormone may contribute to disease severity. This is particularly true because DHEA treatment resulted in clear improvement in clinical symptoms and a moderate increase in the serum concentration of C1 inhibitor (14). When patients with HAE did not demonstrate overt inflammation (see IL-6 serum levels and acute phase proteins), reduction in DHEA is certainly not because of inflammation as in other diseases (15). Furthermore, patients did not receive any further medication which might interfere with DHEA secretion.

Dehydroepiandrosterone is an important prehormone for testosterone production (Fig. 1). This prompted us to study free testosterone in relation to the precursor DHEA. This ratio was not different between healthy subjects and HAE, but it is obvious that danazol treatment largely increased this ratio irrespective of gender. In addition, serum free testosterone levels were increased in patients under danazol treatment. We do not think that a change in levels of sex hormone binding globulin (SHBG) plays a big role for this effect because danazol was administered for a long time in our patients, which leads to near normalization of SHBG levels (16). As we measured serum levels of free testosterone, this investigation was independent of SHBG (17).

It has been demonstrated that danazol inhibits aromatase expression in endometrial explants (18). Similarly,

danazol treatment of women with breast cancer decreased serum levels of oestrone levels (19), and danazol inhibited oestrone formation from cultured breast cancer cells and human granulosa cells (20, 21). Thus, it seems that the increase in testosterone is because of inhibition of the aromatase. In this study, this is corroborated by the fact that 17β -oestradiol was very low in relation to free testosterone in patients treated with danazol irrespective of gender. An unfavourable role of 17β -oestradiol has been demonstrated by others because serum levels of this hormone were correlated to more oedematous attacks (17). Now the question remains how androgens such as testosterone are able to modulate symptoms in HAE patients.

It has been demonstrated that androgens such as testosterone and DHEA can upregulate C1-INH from monocytic and hepatoma cell lines (9). However, this issue has been controversially discussed (22). From our study, high androgen levels are associated with elevated levels of C2 and C4 as shown in Table 2. Another study demonstrated that testosterone may prevent complement mediated lysis of haematopoietic cells (23). As bradykinin is involved in the pathophysiology of HAE, inhibition of bradykinin-mediated effects may be additionally favourable in these patients. Indeed, testosterone can inhibit bradykinin-induced intracellular calcium kinetics in rat aortic endothelial cells in culture (24). Testosterone inhibits bradykinin-induced relaxation of coronary artery rings (25). These different effects illustrate how testosterone might inhibit oedema symptoms in patients with HAE. The fact that serum levels of testosterone are normal in patients not treated with danazol does not rule out increased conversion of DHEA to testosterone on the local cellular level.

In conclusion, we observed decreased serum levels of DHEA in patients with HAE irrespective of danazol treatment. Under consideration of favourable effects of androgens on pathophysiological components of angioedema, the observed decrease in DHEA may be a prerequisite for angioedema formation. This study further demonstrated that danazol in patients with HAE strongly increased testosterone in relation to precursors and downstream oestrogen. Danazol treatment in HAE may decrease oedema formation by increasing highly active androgen testosterone, thus, interfering with several pathophysiological pathways of local oedema formation.

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