Effects of age and gender on pituitary–adrenocortical responsiveness in humans

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This study compared plasma concentrations of adrenocorticotropic hormone (ACTH) and cortisol in young men (N = 10, mean age 24.4 years), young women (N = 10, mean age 25.4 years), old men (N = 8, mean age 81.6 years) and old women (N = 8, mean age 83.5 years) under basal resting conditions and after stimulation with either human corticotropin-releasing hormone (hCRH, 100µg iv) or a combined injection of hCRH (100µg) and arginine vasopressin (VP, 0.5 IU iv). Basal secretion of cortisol did not differ among groups, but basal concentrations of ACTH were diminished in young women (p < 0.01), indicating an enhanced adrenal sensitivity to ACTH in these subjects. Pituitary responses to hCRH did not differ between young men and women. However, responses to hCRH/VP were stronger in the young females (p < 0.01), suggesting an enhanced pituitary responsiveness to the augmenting effect of VP on ACTH release in this group. Pituitary–adrenal secretory responses were greater in old than in young men after sole injection of hCRH (p < 0.05) and even more so after combined injection of hCRH/VP (p < 0.01). In old women, pituitary–adrenal secretory responses were also greater than in young women (p < 0.05). But, in particular for responses to hCRH/VP, these effects were less distinct than within the men. Results indicate an enhancing effect of age on pituitary responsiveness to the hypothalamic secretagogues hCRH and VP, modulated by the subject’s gender.

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Aging appears to be accompanied by a progressive disturbance of the hypothalamus–pituitary–adrenal (HPA) system, which is the most important mediator of the neuroendocrine response to stress (1). Glucocorticoid concentrations have been shown to increase with age in various animal species, during basal resting conditions and after exposure to stress (2–7). Explanations of the age-dependent hypersecretion of glucocorticoids in experimental animals have focused on the role of hippocampal corticosteroid receptors for the feedback regulation of HPA secretory activity (8, 9). However, there is growing evidence from animal studies that age-dependent dysfunctioning of the HPA system is not restricted to the hippocampus and hypothalamus but occurs also at the pituitary level (e.g. 4). Also, two human studies (10, 11) have reported on a tendency towards enhanced peak ACTH and cortisol concentrations following administration of ovine CRH in healthy elderly compared to younger controls. However, these changes were not confirmed by two other studies (12, 13).

Contrasting with the rather inconsistent findings after stimulation solely with CRH, substantially elevated responses of ACTH and cortisol were found in the healthy elderly when stimulated with a combined bolus injection of human CRH (hCRH) and vasopressin (VP) (14). Vasopressin is, besides CRH, the most important secretagogue for ACTH (15–17). Hence, the combined administration of both peptides appears to be a more physiological test of pituitary–adrenal functioning. Thus, one reason for the substantial age-related elevation of pituitary–adrenal responsiveness observed by Dodt et al. (14), which was not found to this degree in other studies (10, 11), could have been the addition of VP.

The effect of gender on pituitary–adrenal activity is even less well investigated than that of age, although a distinct dependency on the sex is well known for the release of some other pituitary hormones (18). Studies in rats and humans have suggested a greater sensitivity of the adrenals to ACTH in females than in males (19–22). Thus, in humans cortisol secretion during the 24-h cycle did not differ significantly between the sexes, but ACTH secretion was higher in men than in women (18). Secretory responses to administration of hypothalamic secretagogues of ACTH so far have not been compared appropriately between the sexes.

The present study aimed to distinguish the effects of age and gender on pituitary–adrenocortical responsiveness to hCRH alone and to a combined injection of hCRH and VP.
Subjects and methods
Sixteen physically and mentally healthy elderly (eight females, eight males) and 20 healthy young subjects (10 females, 10 males) participated voluntarily in the study. Age was comparable for females and males of the aged (female: mean 83.5 years, range 72–91 years; males: mean 81.6 years, range 75–91 years) and of the young controls (females: mean 25.4 years, range 21–31 years; males: mean 24.4 years, range 22–28 years). The aged subjects were recruited by advertisements (n = 7) and from inpatients of the Department of Internal Medicine who were admitted for diagnostic procedures that were completed at least 2 days before the experimental tests (N = 9). The young controls were students or belonged to the hospital staff.

Subjects were screened by history, physical examination and laboratory testing (including routine blood count, measurement of serum electrolytes and urine analysis). Body weight was within the normal range and averaged (+ SEM) 76.9 ± 2.0 kg in the group of young men, 63.3 ± 2.1 kg in the young women, 73.1 ± 2.9 kg in the aged men and 70.9 ± 4.1 kg in the aged women. None of the subjects took medications known or suspected to affect HPA functioning. Subjects with a history of alcohol abuse (exceeding 2 oz/day), with central nervous diseases (complete stroke, transitory ischemic attacks, insufficiency of cerebral arteries, infarcted cerebral areas, dementia, etc.), with severe coronary artery disease and with a history of renal failure were excluded. The young women had normal spontaneous menstrual cycles. The self-reported length of the cycle prior to experiments was between 27 and 33 days. None of the women had taken oral contraceptives for at least 5 months. All participants gave written informed consent. The study was approved by the local ethics committee.

Procedure and design
Each subject participated in two experimental sessions that were at least 2 days apart. Sessions started at 16.00 h, with the insertion of a catheter into an antecubital vein, and lasted until 19.00 h. Throughout the experiment, NaCl solution (0.9%) was infused slowly at a constant rate. During the tests, subjects were awake and rested in a supine position.

Blood samples were collected at 16.00, 16.30 and 17.00 h to determine basal plasma concentrations of ACTH and cortisol. At 17.00 h, each subject was injected with a single intravenous bolus injection containing either hCRH (100 μg; Bissendorf Peptide, Wedemark, Germany; dissolved in 1 ml of saline solution) alone or a combination of hCRH (100 μg) and arginine vasopressin (0.5 IU; Pitressin, Park-Davis, Berlin, Germany). Further blood samples were drawn 15, 30, 45, 60, 90 and 120 min following injection of the peptides. Injections of hCRH and hCRH/VP were well tolerated by all subjects.

The experiments were held double blind. The order of treatments was balanced across subjects. In the young women, stimulation with hCRH took place during menses in three cases, preovulatory in three cases and during the luteal phase in four cases. Combined stimulation with hCRH/VP took place during menses in three cases, preovulatory in four and luteally in three cases.

Assays and statistical analyses
Plasma ACTH was determined immunoradiometrically (Allegro HS ACTH, Nichols Institute, Bad Nauheim, Germany). Plasma cortisol was determined by an enzyme-linked immunosorbent assay (Enzymtest Cortisol ES 300, Boehringer, Mannheim, Germany). Assay sensitivities were 1 pg/ml for ACTH, and 1 μg/dl for cortisol. The intra-assay coefficients of variation were <3% between 5 and 100 pg/ml for the ACTH assay and <5% between 1 and 50 μg/dl for the cortisol assay. Interassay coefficients of variation were below 8%. Samples from a subject were analyzed in duplicate in the same assay.

Effects of age and gender on basal concentrations of ACTH and cortisol (prior to injection of hCRH and hCRH/VP) were evaluated by analyses of variance (ANOVA), including group factors for age and gender and repeated-measures factors for occasion (first vs second) and time (−60, −30 and 0 min prior to injection). Comparisons of stimulated ACTH and cortisol release relied on analyses of covariance (ANCOVA), including the hormonal concentration at 0 min as covariate. The factors were age, gender, treatment (hCRH vs hCRH/VP) and time (15, 30, 45, 60, 90 and 120 min postinjection). Contrasts were calculated to determine differences in hormonal concentrations among any two experimental conditions, separately for each point in time. Analyses of variance were also computed for peak concentrations and the areas under the curve between 0 and 120 min postinjection (with reference to preinjection baseline concentrations at 0 min). A Greenhouse–Geisser-corrected p value of <0.05 was considered significant.

Because the body weight differed significantly between the groups of young men and women (but not between any other two groups), all of the above-described analyses were run with an additional covariate for body weight. However, because these analyses did not indicate any effect of body weight on plasma cortisol and ACTH concentrations, the present report was restricted to results from analyses not including this covariate.

Results
Preinjection basal plasma concentrations of cortisol
were not affected by age and gender. However, basal concentrations of ACTH were lower in young women, when compared to those in young men (p < 0.025) and also in old women (p < 0.01; Table 1). Stimulation with hCRH and with hCRH/VP in all groups invoked a pronounced increase in plasma ACTH and cortisol concentrations. The addition of VP accelerated the rise in ACTH and cortisol concentrations, so that 15 and 30 min following injection of hCRH/VP these concentrations were higher than following injection of hCRH alone (p < 0.001, for main effects of treatment across experimental groups). Moreover, the postinjection increase in ACTH and cortisol concentrations, in general, was more pronounced in the aged than in young controls, and also more pronounced in female than in male subjects (p < 0.01 for main effects of age and gender).

However, the effects of treatment, age and gender strongly interacted. Effects of age depended significantly on the subject’s sex, and vice versa. Both effects of age and gender, in addition, were modulated significantly by the type of secretagogue administered. This is specified in the following.

**Effects of gender**

Unlike in young women, in young men the injection of hCRH alone induced a maximum secretory response of the pituitary–adrenal system (Table 1). Addition of VP did not increase the secretory response but only shortened its latency. Thus, comparing effects of stimulation in young men with those in young women indicated greater secretory responses of cortisol and ACTH in the female than in the male subjects, but only when stimulated with hCRH/VP. Responses of cortisol and ACTH after stimulation with hCRH alone did not differ between the sexes, except that towards the end of the recording epoch (90 and 120 min post-injection) the cortisol concentrations remained slightly elevated in the young women.

Peak responses of cortisol and ACTH and respective areas under the curve were also higher in aged women than in aged men. Yet, unlike in the young subjects, in the aged this difference reached significance only following injection of hCRH alone, but not following injection of hCRH/VP (Table 1). Moreover, in the elderly subjects, the effect of gender after injection of hCRH appeared to be more variable, reaching only marginal statistical significance when evaluated for single points in time after injection.

**Effects of age**

Comparing stimulated cortisol and ACTH concentrations between young and old men (Fig. 1a and Table 1) indicated a greater responsiveness of the pituitary–adrenal axis in the aged men. After combined stimulation with hCRH/VP, the effects on cortisol and ACTH started earlier and—as indicated by analysis of peak concentrations and areas under the curve—were also stronger than after injection of hCRH alone (p < 0.05 for respective treatment and age

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Table 1. Preinjection baseline concentrations (−30 to 0 min), peak concentrations and areas under the response curve (0–120 min) following injection of human corticotropin-releasing hormone (hCRH, 100 μg) and a combined injection of hCRH (100 μg) and arginine vasopressin (VP, 0.5 IU) in young (N = 10) and aged men (N = 8) and young (N = 10) and aged women (N = 8).

<table>
<thead>
<tr>
<th></th>
<th>Baseline (mean ± SEM)</th>
<th>Peak (mean ± SEM)</th>
<th>Area (mean ± SEM)</th>
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<tbody>
<tr>
<td>ACTH</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Young men</td>
<td>CRH</td>
<td>18.6 ± 1.9</td>
<td>24.8 ± 4.4</td>
</tr>
<tr>
<td></td>
<td>CRH/VP</td>
<td>21.3 ± 3.2</td>
<td>24.7 ± 4.3</td>
</tr>
<tr>
<td>Young women</td>
<td>CRH</td>
<td>13.2 ± 1.3</td>
<td>29.9 ± 5.3</td>
</tr>
<tr>
<td></td>
<td>CRH/VP</td>
<td>13.2 ± 1.3</td>
<td>59.9 ± 11.3</td>
</tr>
<tr>
<td>Old men</td>
<td>CRH</td>
<td>18.5 ± 2.2</td>
<td>42.0 ± 6.7</td>
</tr>
<tr>
<td></td>
<td>CRH/VP</td>
<td>18.3 ± 2.5</td>
<td>63.5 ± 13.6</td>
</tr>
<tr>
<td>Old women</td>
<td>CRH</td>
<td>19.7 ± 1.5</td>
<td>87.9 ± 16.8</td>
</tr>
<tr>
<td></td>
<td>CRH/VP</td>
<td>19.4 ± 2.3</td>
<td>101.7 ± 17.0</td>
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</table>

a p < 0.05 and *p < 0.01 for a comparison with corresponding value in young men.

b p < 0.05 and *p < 0.05 and *p < 0.01 for a comparison with corresponding value in young women.

† p < 0.05 and *p < 0.01 for a comparison with corresponding value in old men.
interactions). Nevertheless, ACTH peak concentrations, areas under the response curves for ACTH and cortisol (Table 1) and comparisons for single points in time after injection of hCRH confirmed a significantly stronger pituitary–adrenal response in the aged men (compared to young women) also for this stimulation. After both injection of hCRH/VP and hCRH, ACTH and cortisol secretory responses were prolonged in the aged compared to the young men (Fig. 1a).

Also, in aged women, cortisol and ACTH secretory responses were higher than in young women (Table 1 and Fig. 1b). However, with respect to cortisol, this effect was restricted to stimulation with hCRH alone, and was virtually absent for combined stimulation with hCRH/VP. With respect to ACTH, the effects of age on areas under the curve were, similarly, stronger after injection of hCRH than after hCRH/VP (p < 0.05 for treatment and age interaction). Nevertheless, the effect of age after hCRH/VP on ACTH peak concentrations reached significance, and the related increase in the area under the response curve approached significance (p < 0.08). However, when assessed for single points in time (Fig. 1b), the more pronounced increase in ACTH concentrations in old than in young women reached significance only after a sole injection of hCRH.

Discussion
The release of ACTH and cortisol was evaluated in aged and young men and women prior to (basal secretion) and after stimulation with hCRH and a combined injection of hCRH and VP.

Gender had a significant influence on basal ACTH secretory activity. While cortisol concentrations were comparable for both sexes, basal ACTH concentrations were higher in young men than in women, indicating an increased responsiveness of the female adrenal cortex to ACTH (19, 22). However, this sex difference disappeared in aged subjects, i.e. beyond the menopause, suggesting that the increased adrenal sensitivity to ACTH in young women depended on the presence of high concentrations of gonadal hormones (20, 21, 23).
There were also substantial differences between the sexes in the pituitary–adrenal response to CRH and CRH/VP. In women, the increase in cortisol concentration after injection of hCRH was prolonged and recovery of basal concentrations appeared to be delayed. This does not confirm earlier studies, which indicated no significant differences in the response to CRH between both sexes (24, 25). However, in one of these studies (24) CRH was administered at a dose (200 µg) that was twice as high, and age was not matched between the two groups. In the other study (25), cortisol responses were evaluated for only 1 h after CRH injection. Because the increase in ACTH after injection of hCRH in young women was very similar to that in young men, the higher cortisol response in young women further supports the view that adrenal responsiveness to ACTH is increased in this group. An alternative explanation for the longer lasting effect of hCRH on plasma cortisol in young women could be an increased level of corticosteroid binding globulin (26). However, whether the corticosteroid plasma half-life is also longer in the female than the male organism must be doubted (20, 22).

Sex differences in the pituitary–adrenal responses were even more pronounced after combined stimulation with CRH/VP than with hCRH alone, with the women displaying a distinctly higher sensitivity to the augmenting effect of VP on hCRH-induced ACTH release than the men. At present, few data are available regarding an influence of gender on pituitary–adrenal responsiveness to secretagogues other than CRH. Notably, the first report demonstrating a synergistic effect of VP and CRH on the secretion of ACTH and cortisol in humans was based on experiments in young women (27), and only some years later were similar experiments reported on men (17). However, dose and route of administration of the substances differed between those experiments, hindering a direct comparison of results. In the young men of the present study VP, in fact, did not enhance but merely accelerated CRH-induced ACTH secretory peak responses. This was probably due to the small dose of VP administered (28).

In the aged subjects, the difference between the sexes in the ACTH secretory response to hCRH/VP was diminished and remained non-significant. Cortisol responses to hCRH/VP seemed even comparable in aged men and women. One reason for the diminution in sex-dependent differences in the response to hCRH/VP in the aged appeared to be the increasing effect of age on
pituitary–adrenal responsiveness to VP in men. Moreover, there was a general and strong increase in responsiveness to sole administration of hCRH in the aged. This increase was particularly pronounced in the elderly women, thus preventing a substantial additive effect of combined administration of hCRH/VP in this group. The difference between the sexes in the responsiveness to hCRH/VP and its fading in the aged is at present difficult to explain. It may be related to an inhibiting effect of testosterone on cortico-stimulating functions (29–31).

Age, independent from gender, had a profound enhancing effect on pituitary–adrenal responsiveness to the secretagogues administered. In women, especially after injection of hCRH/VP, the age-dependent increase was less consistent than in men. This was probably due to the fact that the young women serving as control for this comparison already displayed a rather high sensitivity to the additive effect of VP, and in addition a generally enhanced adrenal responsiveness to ACTH.

Together, the present findings confirm and extend a previous report on a significantly enhanced ACTH and cortisol release in mentally healthy elderly following the combined administration of hCRH and lysine vasopressin (14). They demonstrate that hCRH alone is sufficient to discriminate between aged and young subjects of both sexes on the basis of pituitary responsiveness. Discrepant results from other studies (10, 13) in this context are at present difficult to integrate; factors such as the subjects’ age and mental state, the type of CRH used and the time of day may modulate the pituitary–adrenal response.

In addition to the enhanced peak concentrations in the aged men of the present study, recovery of preinjection basal plasma ACTH and cortisol concentrations was delayed compared to the young men. The delay was visible after both stimulation with hCRH and hCRH/VP, but could not be discerned within the female subjects. These results suggest that, at least in men, an impaired feedback inhibition of pituitary–adrenal secretory activity contributed to the age-induced increment in pituitary–adrenal responsiveness (4, 9, 32).

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