CLINICAL STUDY

The adrenal gland may be a target of LH action in postmenopausal women

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Abstract

Objective: LH receptor expression and function have been demonstrated in the human adrenal cortex, but their involvement in normal adrenal function remains elusive. Because cortisol levels have been reported to be higher in postmenopausal women than in age-matched men, the aim of the present study was to investigate a possible association of adrenal function with the elevated LH levels in postmenopausal women.

Design and methods: A group of 112 endocrinologically normal postmenopausal women (mean age 67.6, range 50–88 years) was evaluated. A basal fasting morning sample of peripheral blood was taken for the determination of LH, cortisol, dehydroepiandrosterone-sulphate (DHEA-S), oestradiol (E2), testosterone, sex hormone-binding globulin (SHBG), insulin and glucose. Information about reproductive function, anthropometric parameters and arterial blood pressure was recorded.

Results: The correlation of LH and cortisol was bimodally distributed, with a significant linear correlation up to the LH level of 41 U/l ($n = 78$, $P < 0.01$), after which the increase of cortisol levelled off. Significant associations were also found between serum DHEA-S and LH levels ($P < 0.05$), as well as between cortisol and testosterone ($P < 0.0001$), but not between E2 and LH. Multivariate analysis showed that the association of cortisol with LH was independent of age and testosterone; the association of DHEA-S with LH was independent of E2, cortisol and age. Significant associations were also found between E2, testosterone and DHEA-S levels ($P < 0.001$).

Conclusions: These results indicate that adrenal cortisol and DHEA-S production may be stimulated by the highly elevated postmenopausal levels of LH; the physiological significance of this association and plausible contribution to the metabolic syndrome observed after the menopause remain to be evaluated.

European Journal of Endocrinology 154 875–881

Introduction

In recent years several reports have appeared on the presence of luteinising hormone (LH) receptors in the adrenal cortex, both in humans and several mammalian species (1–7). LH-responsive tumours in non-adrenocorticotrophin (ACTH)-dependent Cushing's syndrome have been identified (8–13) and cases of pregnancy-associated ACTH-independent Cushing's syndrome have been reported (14, 15), presumably caused by ectopic expression of LH receptors responding to human chorionic gonadotrophin (hCG). Moreover, 'thecal metaplasia' in the adrenal gland in both sexes in conditions with elevated gonadotrophin levels is a well known but poorly investigated condition (16, 17). It is not known whether the apparent LH responsiveness of the adrenal gland only represents a rare phenomenon associated with neoplastic processes or whether it also occurs under normal circumstances, such as after the

menopause, and whether chronically elevated LH, as in polycystic ovary syndrome (PCOS) or hypergonadotrophic hypogonadism, could contribute to the stimulation of adrenal steroid production.

A plausible effect of LH on adrenal function would be expected to be relatively minor and difficult to verify under normal circumstances, as ACTH is undisputedly the major stimulus of adrenal steroid production (cortisol and dehydroepiandrosterone sulphate (DHEA-S)). We hypothesised that such a putative direct LH effect on the adrenal gland would become more pronounced in conditions of chronic LH excess. We therefore investigated whether associations can be detected between the serum levels of LH and adrenal steroids in a group of postmenopausal women where, owing to the lack of ovarian oestrogen feedback, the LH levels are chronically increased. The possibility that such adrenal stimulation might contribute to the metabolic syndrome observed at menopause was also considered.

Subjects and methods

We studied a group of 112 postmenopausal women, with a mean age of 67.6 ± 8 (range 50–88) years, who were undergoing evaluation of possible coronary artery disease in the cardiology department. Thirty six of these women had type 2 diabetes mellitus (DM2); six of them were treated with insulin and the remaining 30 with oral hypoglycaemic agents. Seven women had mildly elevated serum creatinine values; two of them had elevated urea levels as well. None of them had known renal or liver disease. Liver function tests were normal in all patients. None of the women were receiving hormone replacement therapy (HRT) or opiates, corticosteroids or antiepileptic drugs. After overnight fast, a peripheral blood sample was taken between 0800 and 0900 h for the determination of serum LH, cortisol, DHEA-S, oestradiol (E2), testosterone, sex hormone-binding globulin (SHBG), insulin, glucose and lipid levels. Information about age at menopause, parity and reproductive function was taken. Weight, height, waist and hip perimeters were measured, and systolic and diastolic arterial blood pressure was recorded.

Biochemical parameters (glucose, creatinine, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides, uric acid) were measured immediately. Serum samples were kept frozen at -20°C until hormone analyses, which were performed at the end of the study, using consecutive batches of reagent kits. E2 was measured using the Spectria E2 sensitive RIA method (Orion, Espoo, Finland), SHBG was determined by IRMA (Radim SpA, Via del Mare, Domecia [Rome], Italy), and serum insulin was determined by IRMA (Biosource Europe SA, Nivelles, Belgium). Total serum testosterone was measured by RIA (Biosource Europa SA), with a reference range of 0.35–2.8 nmol/l, inter-assay CV=6.2% and intra-assay CV=4.6%. DHEA-S was measured by RIA (Radim SpA), with a reference range for menopausal women of 0.3–2.2 $\mu\text{mol/l}$, inter-assay CV=9.7% and intra-assay CV=9.6%. Cortisol was measured by RIA (Immunotech SA, Marseille, France) (reference range at 0800–0900 h: 190–610 nmol/l), inter-assay CV=5.8% and intra-assay CV=6.9%. LH was measured by IRMA (Diagnostic Systems Laboratories, Cherwell Innovation Centre, Upper Heyford, Oxon, UK) with a postmenopause reference range of 9.1–58.3 U/l, inter-assay CV=8.9% and intra-assay CV=6.8%.

The basal insulin resistance index (HOMA) was calculated according to the formula: Insulin

resistance= $\text{FI} \times \text{G} / 22.5$, where FI=fasting insulin (mU/l) and G=fasting glucose (mmol/l).

Statistical analysis

All descriptive data are expressed as mean $\pm$ S.E.M. Statistical analysis was performed using the SPSS statistical package (SPSS Inc., Chicago, IL). Linear regression analysis was used to investigate correlations between the various biochemical and hormonal parameters. The interaction between LH, the adrenal and sex steroids and age was evaluated by multiple stepwise regression analysis. ANOVA was used as appropriate.

Results

The age range of the subjects was 50–88 (median 68) years, the waist/hip ratio was 0.71–1.1 (median 0.92), the body mass index (BMI) was 15.2–44.4 (median 28.1) kg/m^2 , age at menopause was 28–61 (median 49) years, and the time since menopause was 0.5–43 (median 20) years. Mean parity was 2.25 ± 1.35 births (range 0–9), mean height was 157.8 ± 5.8 cm, and mean weight was 72.7 ± 14.2 kg.

The mean values and ranges of the hormonal data are shown in Table 1.

There was a tendency for an increase in cortisol levels with age ($r = +0.21$, $P = 0.07$). There was also a positive linear correlation between cortisol levels and age at menopause ($r = +0.253$, $P = 0.025$). However, multivariate analysis showed that the latter difference was no longer significant when the effect of current age was also taken into account ($P = 0.07$). No association was observed between cortisol and time since menopause, either in the whole cohort or the subgroup with $\text{LH} < 41$ IU/l.

There was a non-significant correlation between serum LH and cortisol, and LH and DHEA-S concentrations. The observed association between the LH and cortisol concentrations showed a tendency for a bimodal distribution, the most probable cut-off point separating the two distributions being at an LH level of 41 IU/l (Figs 1 and 2). The LH/adrenal steroid associations were thus calculated separately for women with LH levels ≤ 41 ($n = 78$) and those with LH levels > 41 IU/l. In the group with the lower LH levels, there was a significant positive linear correlation between LH and cortisol ($r = +0.291$, $P < 0.01$) and LH and DHEA-S ($r = +0.25$, $P = 0.03$; Figs 1 and 2). Above

Table 1 Mean hormonal values in the group of postmenopausal women.

	SHBG (nmol/l)	LH (IU/l)	Testosterone (nmol/l)	E2 (pmol/l)	Cortisol (nmol/l)	DHEA-S (mol/l)
Mean	44.5	33.4	2.02	43.6	466	1.60
S.E.M.	1.93	1.80	0.1	3.5	21	0.09

Figure 1 Correlation of LH with morning serum cortisol levels in postmenopausal women undergoing coronary angiography. Left: significant correlation in the group with $\text{LH} \leq 41 \text{ U/l}$ ($P < 0.01$). Right: no correlation in the group with $\text{LH} > 41 \text{ U/l}$ (broken line indicates non-significant association).

this concentration the steroid levels tended to level off with no further increase parallel to the LH increase. Step multivariate analysis showed that the association of cortisol with LH remained significant when testosterone, age and DHEA-S levels were taken into account (Table 2). The association of DHEA-S with LH remained significant when age, E2 and cortisol levels were taken into account. No significant associations were found between LH and the levels of E2 or testosterone.

There was a highly significant positive linear correlation between cortisol and testosterone levels, which remained highly significant when data across the whole range of LH levels were included in the analysis (Fig. 3). No correlations were found between cortisol and DHEA-S levels either in the whole group or in that with LH levels $\leq 41 \text{ U/l}$. E2 levels correlated significantly with those of testosterone and DHEA-S ($r = +0.512$ and $r = +0.352$, respectively, $P < 0.001$). Testosterone levels had a highly significant correlation with DHEA-S levels ($r = +0.53$, $P < 0.0001$). The SHBG levels did not correlate with those of LH, testosterone, E2 or DHEA-S. There was a highly significant negative linear correlation between DHEA-S and age ($r = -0.391$, $P < 0.001$). Considering the possibility that the women undergoing angiography were under anticipatory stress, it is noteworthy that 18 of them were found to have cortisol levels above the upper limit of the normal range (610 nmol/l), whereas the remaining 94 had cortisol within the normal range. However, the mean LH levels were similar in women with normal and elevated cortisol (32.2 ± 12.8 vs $33.3 \pm 19.9 \text{ U/l}$, not significant). It is therefore unlikely that our observations reflected a stress effect. Furthermore, to explore the possibility that the LH levels could be directly influenced by stress, we compared the distribution of LH concentrations with a group of unstressed postmenopausal women matched for age ($n = 48$) who were attending the outpatient clinic over the same time period. There was no difference in LH between the two groups (32.5 ± 12.5 vs $33.2 \pm 19.1 \text{ IU/l}$, not significant). Seven patients had relatively low LH levels ($< 9.5 \text{ nmol/l}$).

These were all menopausal and > 60 years of age (age range 65–78). Four of them had relatively low cortisol levels; this may indicate that in these cases a small degree of pituitary dysfunction was present. However, our results did not change when these patients were excluded from the analysis.

There were no associations between either cortisol or DHEA-S levels and the waist/hip (W/H) ratio, insulin levels or the HOMA basal insulin resistance index. BMI did not correlate with any of the hormonal parameters that were examined, either in the whole group or in the subgroup with $\text{LH} < 41 \text{ IU/l}$. No associations of BMI with cortisol and LH were found when various cut-off points for BMI were used. Total testosterone tended to have a negative association with the W/H ratio ($r = -0.23$, $P = 0.04$), while no associations with basal insulin resistance index (HOMA) were found. Similar associations were found on all occasions when patients with DM2 were excluded from the analysis. There was a marginally significant association between W/H ratio and age ($r = 0.177$, $P = 0.051$).

Discussion

Our findings show a positive correlation between circulating cortisol and LH levels in postmenopausal women. Moreover, this association appeared to be independent of age and levels of other steroids. Although this observation does not provide direct evidence for LH stimulation of adrenal function, it suggests that this may be the case. Further support for this possibility was provided by a similar positive correlation of LH with DHEA-S, a steroid also of adrenal origin. Adrenal specificity for this finding was provided by the lack of such correlation between LH and E2 levels.

There is considerable evidence that the adrenal gland is responsive to gonadotrophin stimulation in specific pathological cases, such as tumours. The expression of LH receptors in adrenal tissue has been reported by Lacroix *et al.* (4) in adrenal neoplasms producing

Figure 2 Correlation of LH with DHEA-S levels in postmenopausal women undergoing coronary angiography. Left: significant correlation in the group with LH ≤41 U/l ($P<0.05$). Right: no correlation in the group with LH > 41 U/l (broken line indicates non-significant association).

glucocorticoids. These tumours were even shown to be dependent on LH action, since on some occasions glucocorticoid production could be reduced by treatment with the gonadotrophin-releasing hormone (GnRH) agonist leuprolide (18). Similarly, cases of pregnancy-associated Cushing’s syndrome have been reported, again due to responsiveness of adrenal cells to LH/hCG (14, 15). However, adrenal LH responsiveness may not be limited to rare pathological conditions. LH receptor expression has been reported in the normal human adrenal gland (1). The circulating cortisol concentrations are slightly but significantly higher in postmenopausal women than in age-matched men (19), although this is not a constant finding in all reports (20). Furthermore, the ageing-related decline in DHEA-S, which was confirmed in this study, is steeper in men than women (19). At perimenopause, the decreasing tendency in DHEA-S concentrations reverts to an increase parallel with the increase in gonadotrophin levels (21). Finally, an adrenal component in the increased androgen levels of PCOS has been discussed, although not proven, for a number of years (22). Piltonen *et al.* (23) recently tested adrenal responsiveness to acute administration of hCG in women after medical gonadectomy by GnRH agonist, but found no response. The explanation for this lack of effect may be the acute nature of the experiment and that chronic gonadotrophin stimulation, probably for years, may be needed for the induction of expression of a functionally significant level of LH receptors.

Animal experiments show convincing evidence for LH responsiveness of the adrenal gland. Exposure of guinea pig adrenal cells to hCG result in stimulation of cortisol secretion *in vitro* (24). Data from transgenic

mice with chronic elevation of LH also show LH receptor expression in the adrenal cortex and an LH-stimulated increase in corticosterone production (2, 3). The ferret is another mammalian species that has been shown to have clear adrenal steroidogenic response to the high post-gonadectomy levels of gonadotrophins (5).

The adrenal cortex and gonads have an intimate ontogenic relationship. Lineage tracing studies on cells positive for the transcription factor SF-1 have located in the developing urogenital ridge cells forming the adreno-gonadal primordium and subsequently differentiating into two distinct populations, which ultimately become the adrenal and gonadal primordia (25, 26). The gonadal primordium develops into testis or ovary, the adrenal primordium into adrenal cortex. Common cellular origin of the adrenal gland and gonad is also indicated by the ambiguous expression of adrenal and gonad-specific genes in the foetal period. The adrenal-specific P45011 β is expressed in foetal gonads, and the gonad-specific P450arom and P45017 α are expressed in foetal adrenal glands (25). Likewise, the ACTH receptor is expressed in mouse foetal but not adult testis (27). The LH receptor is expressed in mouse foetal but less clearly in adult adrenal gland (28), and human foetal adrenal gland has been shown to respond to hCG stimulation (29). The ambiguity of adrenal and gonad-specific gene expression is lost in adult life, but it may revert back in special situations such as during tumourigenesis. This may occur in gonads in the presence of high ACTH levels (Nelson’s syndrome) (30–33) and in the adrenal gland in the presence of high gonadotrophin levels (after gonadectomy or menopause) (16, 17, 34). Adrenal rest tumours in the gonad are assumed to arise from ACTH stimulation of

Table 2 Stepwise multiple regression model for predicting cortisol levels in postmenopausal women.

Variable	Predictor	Coefficient beta	T	Significance (P)	Overall r^2 (%)
Cortisol	Testosterone	11.5	4.72	0.0000	0.33, $P=0.0000$
	LH	0.234	2.79	0.0067	
	Age	0.282	2.69	0.0089

Figure 3 Association of testosterone with cortisol in postmenopausal women ($r = +0.457$, $P = 0.0001$).

adrenal tissue sequestered in the gonad during embryonic development, but it is also possible that they arise from undefined common adreno-gonadal stem cells in response to high ACTH stimulation. By analogy, adrenal hyperplasia, or thecal metaplasia in the adrenal gland, may originate from a similar stem cell population in response to high gonadotrophin stimulation. The postmenopausal state with chronic elevation of gonadotrophins may represent a condition where the putative stem cells in the adrenal cortex respond to elevated gonadotrophin stimulation with differentiation of a population of LH-responsive steroidogenic cells.

We may speculate that the putative cortisol and DHEA-S stimulating effect of LH in adrenal glands can contribute to the increased frequency of the metabolic syndrome in peri- and postmenopausal women (35). We did not find any associations of LH levels with indices of the metabolic syndrome, such as the waist to hip ratio or the HOMA insulin resistance index; however, the group studied was relatively small and probably underpowered for such a finding.

The reported association of LH with cortisol and DHEA-S could be observed only within a certain window in LH levels and not in women with the highest LH levels, where the cortisol and DHEA-S concentrations appeared to level off. One explanation is desensitisation of the LH effect at the highest hormone levels. Another reason could be that the adrenal effect of LH is saturable and therefore the highest LH levels are unable to bring about further increase in steroid secretion. Despite the fact that both cortisol and DHEA-S were independently correlated to LH, no association between cortisol and DHEA-S was found. This is compatible with the production of the two steroids predominantly in different adrenal zones and

also, possibly, with their different half-lives. The strong association of cortisol with testosterone agrees with other reports in the literature as well as with the notion that the adrenals are the main source of androgens in women after menopause (36).

One point to consider when interpreting these results is the fact that the women were probably stressed at the time of blood sampling before coronary angiography and did not represent the basal situation. In principle, they did not have any obvious reason to show enhanced adrenal responsiveness to LH compared to the non-stressed condition. It is possible, however, that an LH effect on the adrenal gland could have been more marked under conditions of stress where the hypothalamic–pituitary–adrenal axis is stimulated. If this is the case then we may have observed amplification of the LH effect, which in non-stressed individuals would not have been equally prominent. However, this explanation is unlikely because it has recently been reported in postmenopausal women that LH levels are not affected by acute stress in the absence of E2, while they are significantly increased when E2 is substituted (37). Furthermore, the LH levels in our study group did not differ significantly from those measured in non-stressed age-matched healthy controls.

It would have been interesting to examine concomitant ACTH levels in these subjects, but unfortunately this was not possible. However, one might anticipate that any such associations would be more difficult to evaluate owing to the larger variability of ACTH compared to cortisol levels.

In conclusion, our results provide evidence that the adrenal cortex may be responsive to stimulation by the chronically elevated LH levels prevailing in postmenopausal women. It is not known whether LH has similar effects on adrenal function at the low or transiently elevated levels during the menstrual cycle of

premenopausal women, or when it is elevated to a lesser degree as in women with PCOS. In the latter case such action might contribute to the increased DHEA-S production in the subgroup with elevated LH. The common embryonic origin of the adrenal cortex and gonad and the presence of adrenal rest tumours in gonads during elevated levels of ACTH make it a feasible assumption that an analogous induction of gonadotrophin-responsive cells in the adrenal gland could occur during chronically elevated levels of gonadotrophins.

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Received 30 December 2005

Accepted 16 March 2006