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Changes of Adrenocortical Function and the Ovarian Contribution with Age

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Abstract: Dissociation of adrenal cortisol and androgen secretion occurs in elderly men and women. The postmenopausal ovary produces androgens; however, previous reports regarding adrenocortical function in postmenopausal women did not exclude the ovarian contribution to peripheral steroid levels. In the present study, changes in the ovarian contribution to adrenocortical function with age were determined by measuring peripheral concentrations of C_{19} and C_{21} steroids before and 2 weeks after ovariectomy. The rapid adrenocorticotrophic hormone (ACTH) stimulation test (tetracosactide acetate, 0.25 mg i.v.) was performed, and the age-related difference in the response of steroid levels to ACTH stimulation was also determined in ovariectomized reproductive-age (mean age, 33.0 years, $n=6$) and postmenopausal women (mean age, 63.3 years, $n=9$). After ovariectomy, the concentrations of pregnenolone, 17α -hydroxypregnenolone, progesterone, 17α -hydroxyprogesterone, dehydroepiandrosterone, and androstenedione in reproductive-age women decreased significantly to the levels in postmenopausal women. On the other hand, no significant changes in steroid levels before and after ovariectomy were observed in postmenopausal women. When changes in steroid levels in response to ACTH were compared, significant age-related reductions of dehydroepiandrosterone, and androstenedione were observed with age but 4Δ and $45 C_{21}$ steroid levels in response to ACTH were preserved. These results suggest that C_{17-20} lyase activity decreases but 17α -hydroxylase is maintained for preservation of cortisol in response to ACTH. It is known that P450c17 is a single enzyme that catalyzes activity of both 17α -hydroxylase and C_{17-20} lyase. Therefore, changes in adrenal C_{17-20} lyase activity with age might affect adrenal androgen production.

Key words: adrenal cortex, aging, ovariectomy, 17α -hydroxylase, C_{17-20} lyase

Introduction

Aging is associated with many functional changes, e.g., in the cardiovascular system, in bone and lipid metabolism, and in endocrine organs. The effects of estrogen deficiency in postmenopausal women on various tissues has been well documented. In postmenopausal women, the adrenal cortex is believed to be the only source of peripheral sex hormones. Peripheral serum levels of dehydroepiandrosterone sulfate (DHEA-S), the main adrenal androgen, starts to gradually decline in the 20s and 30s; however, there is little change in cortisol levels despite a lack of changes of adrenocorticotropin (ACTH) secretion with age. The discrepancy between levels of adrenal androgens and cortisol and the control mecha-

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nisms of adrenal androgen secretion have been discussed¹⁻³). Vermeulen *et al.* reported the response of plasma levels of C₁₉ and C₂₁ steroids on acute ACTH stimulation in young and elderly men and women⁴). They suggested that the maintenance or increase of $\Delta 4$ steroid secretion in response to ACTH stimulation in elderly persons despite decreases of response of all $\Delta 5$ -steroids might due to the preservation of 3β -hydroxysteroid dehydrogenase (3β -HSD) activity for acute cortisol secretion. Results of our previous study suggest decreases of both $\Delta 5$ and $\Delta 4$ -androgens, DHEA and androstenedione dione (A), in response to ACTH with preservation of the response of 17α -hydroxyprogesterone (17-P) in postmenopausal women strengthens the physiologic role of 17-P. The results also suggest an age-associated decrease in C₁₇₋₂₀ lyase, which catalyzes the transformation of 17α -hydroxylated C₂₁ steroid to the corresponding C₁₉ steroid. However, 3β -HSD activity does not decrease, since 3β -HSD activity is influenced by estrogen in various tissues and removal of estrogen inhibition by menopause maintains 3β -HSD activity in the adrenal cortex.

Although the decline in ovarian function in postmenopausal women causes a marked decrease in estrogen production, the influence of ovarian steroids on adrenal androgen production is not fully understood. The postmenopausal ovary still produce androgens⁵); however, previous studies of age-related changes of adrenocortical function did not exclude the ovarian contribution^{4,6}). Therefore, the present study was designed to clarify a possible effect of the ovary on adrenal steroid secretion in response to ACTH stimulation by comparing the response of peripheral steroid levels after ovariectomy in young and elderly women.

Subjects and Methods

Subjects

The subjects were reproductive-age women with regular menstruation (mean age, 33.0 years [range, 29-39 years], *n*=6) and postmenopausal women (mean age, 63.3 years [range, 52-76 years], *n*=9) scheduled to undergo hysterectomy with bilateral adnexectomy for cervical cancer. Reproductive-age women were endocrinologically healthy, and postmenopausal women were at least 5 years past menopause. Peripheral blood was collected before and 2 weeks after the operation, and steroid levels were measured to compare the effect of the ovary on peripheral steroid concentrations with age. Two weeks after ovariectomy, the rapid ACTH stimulation test⁶) was performed in the same patients to evaluate age-related changes in adrenocortical response to ACTH without the influence of ovarian function. Tetracosactide acetate (Cortrosyn®, Daiichi Pharmaceutical Co., Tokyo) was given intravenously, and blood samples were obtained before and 30 minutes after the injection. Serum samples were kept frozen at -40°C until assayed. The purpose of the study was explained and informed consent was obtained from all patients.

Steroid measurements

Peripheral concentrations of pregnenolone (P_s), 17α -hydroxypregnenolone (17-P_s), progesterone (P), 17-P, DHEA, A, and cortisol were measured by radioimmunoassay with specific antibodies and chromatographic separation with a Sephadex LH-20 microcolumn as previously described^{6,7}).

Statistical analysis

Student's *t*-test and paired *t*-test were used for statistical analysis.

Table 1. Steroid levels before and 2 weeks after ovariectomy in reproductive-age and postmenopausal women.

Steroids (ng/ml)	Reproductive-age women (n=6)		Postmenopausal women (n=9)	
	before	after	before	after
Pregnenolone	1.02±0.12	0.53±0.07*	0.68±0.09	0.64±0.06
Progesterone	0.73±0.04	0.44±0.08*	0.49±0.04	0.42±0.04
17 α -hydroxypregnenolone	1.36±0.11	0.76±0.09*	0.70±0.05	0.67±0.05
17 α -hydroxyprogesterone	0.65±0.08	0.22±0.01*	0.41±0.05	0.30±0.02
Dehydroepiandrosterone	3.89±0.36	1.84±0.13*	1.97±0.24	1.68±0.15
Androstenedione	0.66±0.05	0.39±0.03*	0.45±0.02	0.40±0.03

before vs after (ovariectomy), * $p < 0.01$, mean $\pm$ S.E.

Fig. 1. Changes of steroid levels before and 2 weeks after ovariectomy. Peripheral steroid levels were measured in ovariectomized reproductive and menopausal women by radioimmunoassay.

Results

Changes in steroid levels before and after ovariectomy with age

Changes in steroid levels before and 2 weeks after ovariectomy in reproductive-age and postmenopausal women were determined (Table 1). Significantly higher concentrations of C₂₁ steroids P₅, P, 17-P₅, and 17-P and C₁₉ steroids DHEA and A were observed in re-

Fig. 2. Stimulating effect of ACTH on peripheral cortisol levels in women 2 weeks after ovariectomy. Acute ACTH stimulation test (cortrosyn® 0.25 mg i.v.) was performed to reproductive ($n=6$) and menopausal ($n=9$) women who were ovariectomized. Peripheral venous blood were collected before and 30 minutes after ACTH administration. No significant change of the increment of overbasal levels in cortisol between reproductive and menopausal women was observed.

productive-age women. Steroid levels were significantly ($p<0.01$) decreased to the levels of postmenopausal women by ovariectomy (Fig. 1). On the other hand, no significant changes were obtained before and after ovariectomy in any of the steroids in postmenopausal women.

Response of steroid levels to ACTH stimulation of different ages in ovariectomized women

The rapid ACTH stimulation test was performed in the same patients who underwent ovariectomy 2 weeks earlier, and the differences in response of steroid levels to ACTH stimulation between reproductive-age and postmenopausal women were compared. Increases over basal levels in response to ACTH stimulation were observed (Fig. 2 and 3). There was no difference in the response of cortisol levels to ACTH between reproductive-age and postmenopausal women. No age-associated change in P_5 , $17-P_5$, P , or $17-P$ in response to ACTH stimulation was shown. On the other hand, significant decreases in the responses of DHEA and A to ACTH stimulation in postmenopausal women were observed.

Discussion

Cortisol secretion from the adrenal cortex in response to stress via the hypothalamic-pituitary axis is maintained despite increased age. Basal plasma concentrations of glucocorticoids, measured as immunoreactive cortisol, tend not to change with age, despite a corresponding decrease in the metabolic clearance of cortisol⁽⁸⁾. Neither the amount nor the binding of corticosteroid-binding globulin (CBG) decreases significantly with age⁽⁹⁾. Direct stimulation of the adrenal cortex by ACTH results in normal or slightly prolonged cortisol response in elderly people⁽⁸⁾. In contrast to the minimal effects on cortisol secretion, levels of peripheral C_{21} and C_{19} steroids, including $17-P_5$, $17-P$, DHEA, and A, decrease significantly in postmenopausal women⁽⁶⁾. These steroids are produced by both the adrenal cortex and the ovary, and the concentrations of these steroids in the ovarian vein in postmenopausal women are significantly higher than in peripheral veins^(5,10). Greenblatt et al. reported

Fig. 3. Stimulating effect of ACTH on peripheral steroid levels in women 2 weeks after ovariectomy. Acute ACTH stimulation test (cortrosyn® 0.25 mg i.v.) was performed to reproductive (n=6) and menopausal (n=9) women who were ovariectomized. Peripheral venous blood were collected before and 30 minutes after ACTH administration.

that the postmenopausal ovary produces androgens but no estrogens in response to intravenous administration of human chorionic gonadotropin (hCG)⁵). According to our previous report, steroid concentrations in ovarian venous blood of postmenopausal women were elevated when the acute ACTH stimulation test was performed during gynecologic operations and steroids from the adrenal cortex were elevated in peripheral veins¹⁰). The results support the hypothesis that the postmenopausal ovary participates in the response to peripheral changes in steroid levels.

To our knowledge, this is the first report of age-related changes in adrenocortical steroidogenesis without the influence of ovarian function in women who had undergone ovariectomy. First, the differences in the values of peripheral steroids before and after ovariectomy in reproductive-age and postmenopausal women were demonstrated. The lack of difference in values before and after ovariectomy in postmenopausal women suggests that the ovarian contribution to peripheral steroid levels in postmenopausal women is limited. Therefore, the difference in the response of peripheral steroid levels to acute ACTH stimulation with age was determined. No significant difference in peripheral levels of P₅, P, 17-P₅, or 17-P in response to ACTH stimulation between reproductive-age and postmenopausal women was

observed. Furthermore, a marked response of 17α -hydroxylated C_{21} steroids to ACTH was also obtained. However, it is of interest that the response of C_{19} steroids in postmenopausal women was significantly lower than that in reproductive-age women. Results of the present study indicate the preservation of adrenocortical 17α -hydroxylase activity and a reduction of C_{17-20} lyase activity in postmenopausal women. These findings are in agreement with those of a previous review of the divergence of adrenal androgen and cortisol²⁾. Kamiyama *et al.* measured enzyme activities of the postmenopausal ovary by *in vitro* incubation and found a significant decrease in C_{17-20} lyase but not in 17α -hydroxylase activity of the postmenopausal ovary¹¹⁾. Many reports have documented the dissociation of adrenal androgen and cortisol secretion with age¹²⁾. Since a single enzyme, P450c17, can catalyze both 17α -hydroxylase and C_{17-20} lyase reactions, induction of this enzyme is believed to control adrenal androgen secretion¹³⁻¹⁶⁾. Both DHEA production and P450c17 mRNA expression by the bovine adrenal cortex are less in old animals than in young animals¹⁵⁾. It is known that the testes, a major production site of androgen, possesses more potent C_{17-20} lyase activity than does the adrenal cortex. Results of the current report suggest that the difference in C_{17-20} lyase activity in various tissues and between species might be due to the regulation of electron transport for P450c17 enzyme cytochrome b5^{16,17)}.

Recently, estrogen replacement therapy for postmenopausal women has been widely accepted to prevent osteoporosis and cardiovascular disease. Effects of estrogen withdrawal and replacement therapy on adrenocortical steroidogenesis remain to be elucidated.

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