Evidence for two independent effects of oestradiol benzoate on the renin-angiotensin-aldosterone system

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Abstract. Plasma renin concentrations (PRC), plasma renin substrate concentration (PRS), plasma aldosterone and cortisol concentrations as well as plasma renin activity (PRA) were measured in ovariectomized subjects after im administration of 10 mg oestradiol benzoate (EB).

The esterified oestrogen exerts two independent effects on the renin-angiotensin-aldosterone system.

1) 48 h after EB administration, PRA was significantly increased. Similar results were obtained for total plasma cortisol, reflecting transcortin concentration. In both cases, the increase was quantitatively related to the basal concentrations. These observations are consistent with the well known oestrogen-induced protein synthesis in the liver.

2) The elevation of PRC preceded that of PRS and was already significant 11 h after EB injection. The early rise in plasma renin activity was essentially caused by the increase in PRC, whereas an influence of the activated substrate synthesis was found later, between the 2nd and the 4th day post injection. The time course of plasma aldosterone concentration correlated well with the increased PRA.

The results provide evidence that EB has two different effects on the renin-aldosterone axis: an early one by elevating renin release and a delayed one by increasing renin substrate synthesis. Whereas the second mechanism can clearly be localized in the liver, extrarenal as well as direct renal effects of EB may be responsible for the renin stimulation.

It is generally accepted that oestrogenic steroids interfere with blood pressure and with water and electrolyte homeostasis. A direct antinatriuretic effect of oestrogens in the kidney has already been postulated more than 40 years ago (Thorn et al. 1938). Later reports indicated that the oestrogen-induced salt retention occurs only indirectly (Dignam et al. 1956; Preedy & Aitken 1956; Johnson & Davis 1976). Helmer & Griffith (1952) published experiments showing an increase of renin substrate concentration during diethylstilboestrol treatment in the rat. Laragh et al. (1967) described an increased angiotensin production rate associated with this angiotensinogen change, and Skinner et al. (1969) demonstrated a decrease of plasma renin concentration after previous stimulation of renin substrate synthesis. Since that time, further studies have confirmed an activation of the renin-aldosterone axis by oestrogens and contraceptive steroids. The present knowledge on the interactions between female sex hormones and the RAAS has been reviewed in detail recently (Kaulhausen 1980).

Oestradiol benzoate is a highly effective monoester, pharmaco-kinetically different from the non-esterified polar steroids in its delayed absorption and its prolonged effect (Murad & Gilman 1975). It was the purpose of this investigation to examine...
the influence of oestradiol, administered as EB, on the RAAS after elimination of the main natural hormone source and during exogenous administration in therapeutic doses.

This seemed to be important because of two earlier unexplained findings:

1) during the preovulatory phase of the menstrual cycle, peaks of renin and aldosterone have been demonstrated which can not be explained by changes in progesterone or 17α-hydroxyprogesterone concentrations (Kaulhausen et al. 1978).

2) in an ovariec-tomized patient treated with 5 mg of oestradiol benzoate, plasma renin activity clearly increased without any measurable change in plasma renin substrate concentration (Kaulhausen et al. 1980). Additionally, the renin-renin substrate Michaelis constant (K_m) and maximal velocity (V_max) were increased following administration of ethinylestradiol in normal subjects (McDonald et al. 1977). All these findings strongly suggest an oestrogen-induced acceleration of the enzymatic activity of renin in man.

In the present study, special consideration was given to the simultaneous measurement of the various components of the RAAS in order to examine the time course of the different effects of oestradiol benzoate as well as to identify the primary change. To obtain additional indication for the influence of this oestrogen on hepatic protein synthesis in ovariec-tomized women, plasma cortisol concentrations were also measured. Since transcortin (corticosteroid-binding globulin) is always saturated with cortisol, conclusions can be drawn, under the conditions of known transcortin increase, from total plasma cortisol concentration to the amount of its binding globulin in plasma.

Materials and Methods

Subjects

Fifteen female patients, 31 to 54 years old and referred to the Department of Obstetrics and Gynaecology of the University of Bonn because of benign tumours of the uterus and the ovaries participated in this study. Informed consent was obtained. Kidney, liver, heart or endocrinological diseases and contraindications against oestrogen treatment were excluded by clinical and laboratory examinations. With the exception of prophylactic heparin therapy (16000–20000 IU/day sc), no other medications were given. The anticoagulation was started on the day of operation and was not changed during the study period. Beginning with the 6th day after hysterectomy and bilateral ovariectomy, venous blood was taken to obtain basal values as described later. Following the 3rd blood sampling, an im injection of 10 mg (2 ml) oestradiol benzoate (Progynon®B oleosum, Schering AG, Berlin) was given to 10 subjects (treated group). The other 5 women received injections of 2 ml normal saline (control group). The subjects were not informed whether they belonged to the oestrogen-treated or to the control group. Additional blood samples were taken 11 h as well as 1, 2, 3, 4, 6 and 8 days post injectionem (pi). The patients received no special diet.

From all subjects, blood was taken at 8 a.m. after having been awokened at least 2 h earlier, and laying in bed again 2 h before sampling. Blood was collected into pre-chilled, disodium-EDTA containing monovettes (Fa. Sarstedt, Nümbrecht) and kept in ice-water up to 60 min until centrifugation at 4°C. Aliquots of plasma were kept frozen at −25°C until analysis was performed.

Radioimmunoassays

All parameters were determined by radioimmunological techniques. PRA was measured according to Cohen et al. (1971) by radioimmunoassay of angiotensin I, which was released during incubation for 60 min of the pre-treated plasma samples at pH 5.6. The coefficients of variation were 4.1% (intra-assay) and 7.5% (inter-assay), respectively.

PRS was determined by radioimmunoassay of angiotensin I after incubation for 2 h of the diluted (1:20 vol/vol) plasma samples with an excess of homologous renin, extracted from human kidneys in cooperation with Professor Oelkers, Berlin. The renin preparation contained at least 0.2 U/ml, calibrated against a standard renin provided by the Medical Research Council, London (MRC 68/356). The coefficients of variation were 7.1% (intra-assay) and 9.7 (inter-assay), respectively. The PRA and PRS methods have been described in detail previously (Kaulhausen et al. 1974).

PRC was measured according to Hummerich & Krause (1975). The renin substrate was prepared from serum of nephrectomized sheep; 1 mg of the lyophilisate contained 40 ng angiotensin I-equivalents. The renin used to obtain a standard curve was received from the MRC, London. The plasma samples and the standard renin solution respectively, were incubated with the sheep substrate for 30 min at pH 7.5 and 37°C. The coefficients of variations were 3.1% (intra-assay) and 5.9% (inter-assay).

Plasma aldosterone concentrations were measured radioimmunologically without previous chromatography after a solvent-extraction with 5 ml methylene chloride (Vetter et al. 1973). The coefficients of variation were 6.8% (intra-assay) and 9.4% (inter-assay).

The method of Vecsei et al. (1972) was used to determine total plasma cortisol concentrations. The precipitation of plasma proteins occurred in an extraction step with absolute ethanol. The antiserum (No. 16) was pro-
Fig. 1.

Effects of an im injection of 10 mg oestradiol benzoate (day 0) on the plasma concentrations of renin (PRC), renin substrate (PRS), cortisol (PC) and aldosterone (PA) as well as on plasma renin activity (PRA) in 4 ovariectomized subjects.

vided by Professor Vecsei, Heidelberg. The coefficients of variation were 4.2% (intra-assay) and 6.2 (inter-assay), respectively.

The reagents used in the radioimmunoassays were obtained from Becton, Dickinson GmbH, Heidelberg ([¹²⁵I]angiotensin I, angiotensin I standard and antiseraum for the PRA- and PRS-methods), and from Isotopendienst West, Dreieich (PRC, PA). The radioactive
Mean values and standard deviations of the different parameters of the renin-angiotensin-aldosterone system and of plasma cortisol concentration (PC) in 10 women treated with oestradiol benzoate and in 5 control subjects who did not receive oestrogen (shaded area; the mean values of the controls are demonstrated by the dotted line). The mean values after administration of oestradiol benzoate were compared with the arithmetic mean of the 3 individual basal values before treatment: the statistical significance was tested with the Student's t-test for paired data.

cortisol was received from New England Nuclear, Boston.

The statistical significance of the mean value changes of the different parameters was tested with the t-test for paired data. The results are shown in Fig. 2. The significance of the correlation coefficients (Table 1) was tested after z-transformation according to Fisher (1954).

Results

The effects of oestradiol benzoate on the various parameters of the RAAS in plasma of 4 women are shown in Fig. 1. (The complete curves of the remaining 6 subjects may be obtained on request). The mean values and the standard deviations of the 10 treated and the 5 control subjects as well as the statistical significance of the results are demonstrated in Fig. 2.

Plasma renin concentration (PRC)

In the 10 oestrogen-treated female patients, a significant rise of PRC occurred which was independent of basal values. The increase of PRC persisted until the 4th day pi. On the 6th–8th day
pi, basal levels were reached again. The PRC showed interindividal variations with respect to the time of onset and the degree of its rise, whereas the declining parts were more homogeneous.

*Plasma renin activity (PRA)*

The oestrogen injection also caused a remarkable increase of PRA; its time course can be well represented by the mean value curve (Fig. 2). Mean PRA had doubled already 11 h pi from basal values around 1.0 ng A/1·ml⁻¹·h⁻¹ during the control periods. The average ratio of maximum to control values for individual subjects was 5.6. The absolute increases of PRA were independent of the basal renin activities.

*Plasma aldosterone (PA)*

The EB influence on the aldosterone concentration was less pronounced but could also be demonstrated in all cases. The mean value curve increased after 11 h. As a consequence of the great interindividual variations, the change was not statistically significant, however. A significant rise was found after 24 h pi. Concomitant with PRA, the mean value curve reached its maximum on day 2 and was at basal values again on day 6. On the average, PA was doubled by EB administration, indicating that the extent of PA increase depended on the basal PA level.

### Table 1.

Results of linear regression analysis between the different parameters measured. The statistical significance of the correlation coefficients was tested after z-transformation.

<table>
<thead>
<tr>
<th>Variables</th>
<th>r</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRA – PRS</td>
<td>0.53</td>
<td>n.s.</td>
</tr>
<tr>
<td>PRA – PRC</td>
<td>0.99</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>In PRA – In PRS</td>
<td>0.78</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>PRC/PRA – 1PRS</td>
<td>0.92</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>PRA – aldosterone</td>
<td>0.94</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>PRA – cortisol</td>
<td>0.61</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>PRS – PRC</td>
<td>0.41</td>
<td>n.s.</td>
</tr>
<tr>
<td>PRS – aldosterone</td>
<td>0.49</td>
<td>n.s.</td>
</tr>
<tr>
<td>PRS – cortisol</td>
<td>0.92</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>PRC – aldosterone</td>
<td>0.92</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Aldosterone – cortisol</td>
<td>0.74</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

**Plasma renin substrate (PRS)**

After bilateral ovariectomy, PRS decreased as may be seen from the initial part of the mean value curve (Fig. 2). After EB treatment, an increase occurred with a delay of 1–2 days, continuing thereafter until day 4 pi. On the 2nd day pi, the PRS increase was already highly significant. With the exception of 1 subject, PRS concentrations were doubled. The rising and the declining parts of the summation curve appeared to be symmetrical. The uniformity of the PRS curves in all 10 subjects was remarkable. The investigation period was, however, too short to register the final return of PBS to the original levels.

**Plasma cortisol (PC)**

On the 1st day after EB injection, PC concentrations were unchanged. A statistically significant increase was observed on the 2nd day pi. Maximal values were found on the 3rd day. Comparing the maximal value with the basal value, the relative constancy of the cortisol increase was remarkable, the rising factor ranging between 1.3 and 1.8.

### Different modes of reaction of the renin – aldosterone axis

In 4 subjects, PRC and PRA reached a peak already 11–24 h pi, as demonstrated in 2 women (Fig. 1: subjects Nor. and Ram); the duration of this increase was individually different; however, basal values were generally reached 8 days pi. With one exception (maximum 4 days pi), all other subjects showed maximal values of PRC and PRA 2–3 days pi (Fig. 1: subjects Fis. and Fin.). The degree of PRS and PA stimulation also varied in the 10 subjects; maximal values were mostly observed after 3–4 days.

### RAAS in the control subjects

The im injection of 2 ml normal saline caused no characteristic change of any parameter of the RAAS or of cortisol concentration. Following bilateral ovariectomy, there was a fall of the concentrations of PRS and PC; the same declining tendency of both parameters could also be seen during the control period in the oestrogen-treated subjects.

### Relations between the different parameters of the RAAS

The results of linear regression analysis between the variables are listed in Table 1. The high correlation between PRA and PRC (r = 0.99) confirmed the observation that, without any exception, time

The single injection of oestradiol benzoate (10 mg im) caused, after a delay of 1 day, a doubling of PRS which lasted for at least 7 days. A rise of PRS requires a de novo synthesis of this hepatic protein (Nasjletti & Masson 1972). The time intervals between the hormone injection and the changes of PRS agree well with earlier findings on oestrogen-induced protein synthesis in man (Degos et al. 1978) and in animals (Jackson 1973; Luskey et al. 1974).

In some cases the initial delay observed in the increase of PRS was even more pronounced with cortisol. The high correlation between the PRS and the cortisol curves (r = 0.92) supports the hypothesis that a similar mechanism caused the increase in both cases. In contrast to PRS, the modes of rise and decline of cortisol concentration were different in each individual. Even more apparent than with renin substrate is the dependence of the increase of total PC on the basal levels.

The hypothesis advanced by Laragh et al. (1967), Skinner et al. (1969), Cain et al. (1971) and Beckerhoff et al. (1972) that the PRA increase is only a consequence of an increased renin substrate synthesis could not be confirmed in the present study. Repeatedly, the observation was made that significant increases of PRA had already taken place while renin substrate concentrations were still unchanged (Fig. 1). There were also differences of both parameters with regard to the time of return to basal values: whereas the increased renin activities declined nearly exponentially, PRS concentrations remained elevated until the end of the investigation. In addition to the time shift of both curves, the variability of the initial PRA changes as compared to those of PRS rather contradicted a causal relationship.

Under physiological conditions PRS is rate-limiting for the renin-renin substrate reaction in vitro (Gould & Green 1971; Eggena et al. 1976). The different time courses of the increases in the curves of PRA and PRS presented here however indicate that other factors take part in the stimulation of the RAAS. Furthermore, provided the physiological substrate concentration to be in the range of Km (Krackoff 1973) or to imply 80% of the maximal velocity (McDonald et al. 1977), a huge increase in substrate concentration would at least cause a doubling of PRA. In the present experiment, however, the PRA values were elevated 5.6-fold on average. Thus, the present results show, that, independent of the stimulation of hepatic substrate synthesis, an impressive change of renin release took place after administration of oestradiol benzoate. The inter-individually different rate of PRC and PRA increase (Fig. 1) may be due either to a difference in resorption of oestradiol from the oily im depot or to a variable responsiveness of the renin-producing cells to the oestrogenic stimulus.

Besides extrarenal mechanisms of renin stimulation direct effects of oestrogens on the kidney are probable. An initial natriuresis after administration of high doses of oestradiol, which was described by

Discussion

In pre-menopausal women, bilateral ovariectomy leads to a rapid decrease of plasma oestrogen concentrations; after 6 days, a new plateau is reached on a lower level which is about 1/6 of the pre-operative concentrations (Hunter 1976). In the present study, PRS continued to decline even 6–8 days after ovariectomy in 5 control subjects as well as in the control period of the oestrogen-treated women. This demonstrates the importance of ovarian hormones for the maintenance of normal renin substrate availability. This observation is in agreement with the results of Oelkers et al. (1976), showing a slightly higher normal range of PRS in females compared to males. The simultaneous decrease of total plasma cortisol (PC), which correlates very well with that of PRS (r = 0.99 in the control subjects; Fig. 2), may be explained by the withdrawal of oestrogenic stimulation of hepatic transcortin synthesis. The present findings confirm earlier reports that oestrogens are able to increase renin substrate concentration in plasma (Krackoff 1973; Ménard & Catt 1973; Ménard et al. 1973; Hollenberg et al. 1976; Oelkers et al. 1976; Pallas et al. 1977; Degos et al. 1978).

The single injection of oestradiol benzoate (10 mg im) caused, after a delay of 1 day, a doubling of PRS which lasted for at least 7 days. A rise of PRS requires a de novo synthesis of this hepatic protein (Nasjletti & Masson 1972). The time intervals between the hormone injection and the changes of PRS agree well with earlier findings on oestrogen-induced protein synthesis in man (Degos et al. 1978) and in animals (Jackson 1973; Luskey et al. 1974).

In some cases the initial delay observed in the
Katz & Kappas (1967), would be followed by increased renin secretion.

Lindheimer & Oparil (1977) however could not find any change of sodium excretion or renal haemodynamics during the early phase after injection of oestradiol benzoate. They doubt the validity of Katz’s hypothesis of an initial natriuresis as a physiological event because extremely high doses of oestrogens were given in his experiment. The findings of Lindheimer & Oparil (1977) seem to exclude an interpretation of the present results as a direct haemodynamic or natriuretic effect of oestradiol or EB on the kidney.

Plasma renin reactivity was not determined in the present study. Thus, the theoretical possibility that EB could release an activator or remove a non-competitive inhibitor of renin enzyme activity may not be excluded.

Finally, the possibility that the increase of PRC could be a substance-specific effect of oestradiol benzoate must also be considered. This is strengthened by a comparison of our own results with earlier reports. Evidently, the various oestrogens differ in their qualitative and quantitative ability to activate the different parameters of the renin-aldosterone axis. After administration ethinyl-oestradiol (Oelkers et al. 1976; McDonald et al. 1977; Kaulhausen et al. 1980), plasma renin substrate concentration was the most stimulated parameter; PRA was also elevated but less than demonstrated after oestradiol benzoate in the present investigation. Similar studies in ovariecotomized subjects have therefore been started in our laboratory to examine and compare the effects of oral administration of micronized oestradiol-17β on the RAAS.

Acknowledgments

We thank Dr. W. Hummerich, Köln, for the provision of sheep renin substrate. The cooperation of Prof. Dr. W. Oelkers, Berlin (human renin preparation), Prof. Dr. P. Vecsei, Heidelberg (cortisol antiserum) and of the Medical Research Council, London (standard renin preparation), is gratefully acknowledged. Excellent secretarial assistance was provided by Karin Pfeiffer.

References


Received on May 26th, 1981.