Urinary excretion of relaxin after estradiol treatment of postmenopausal women

T. H. LIPPERT - F. P. ARMBRUSTER (*) - H. SEEGER - A. O. MUECK
M. ZWIRNER - W. VOELTER (*)

Summary: The influence of estradiol treatment on the urinary excretion of relaxin, a hormone in earlier years only found during pregnancy and presently associated with functions in the cardiovascular system, was investigated in postmenopausal women. Thirteen postmenopausal women were treated with transdermal estradiol and 12 women with oral estradiol for 4 weeks. A new radioimmunoassay for human-relaxin (rec-hRLX-2) was used. With transdermal, but not with oral administration, a significant increase of urinary relaxin excretion was registered. Further experiments are necessary to elucidate the source of urinary relaxin and its role in the hormone replacement therapy of postmenopausal women.

Key words: Hormone replacement therapy; Postmenopausal women; Urinary relaxin excretion.

INTRODUCTION

Relaxin, for many years considered only as a pregnancy hormone ([1]), has also been found, with improved detection technique, in non-pregnant women. Recently, experiments in non-pregnant animals indicated that relaxin influences the cardiovascular system by developing chronotropic and inotropic effects as well as vasodilation and the lowering of blood pressure ([2-7]). Thus relaxin may be involved in maintaining systemic circulation.

Little is known about its function in non-pregnant women or men. Recent data have shown a correlation between circulating blood levels of relaxin and estradiol during the human menstrual cycle ([8]). Since estradiol, known for its cardioprotective action in postmenopausal women, causes vasodilation ([9]), it seemed of interest to investigate its effect on relaxin metabolism. By analogy with previous studies ([10, 11]) which investigated the influence of estradiol on the excretion of vasoactive substances relaxin was measured in the urine.

MATERIALS AND METHODS

Postmenopausal women (n. = 25) with climacteric symptoms were enrolled in this study. All the women had ceased menstruating for at least 12 months. Seventeen-beta-estradiol levels in serum were below 70 pmol/l and FSH levels were above 40 IU/l.

Received 6-7-1995 from the
Department of Obstetrics and Gynecology
(*) Department of Physical Biochemistry
University of Tuebingen - Tuebingen, Germany
Revised manuscript accepted for publication 12-9-1995.

Hormone replacement therapy (HRT) was started with unopposed estradiol, 13 women receiving it transdermally (Estraderm TTS, 0.05 mg/day) and 12 women orally (estradiol valerate, 2 mg/day)) for 4 weeks. The study was open and random allocation was not used.

Urine, excreted between 10 pm and 6 am, was collected during two consecutive nights before and after 14 and 28 days of estradiol treatment. The collection of urine during the night provided on the one hand an easy and reliable way of sampling, and on the other hand a concise time period well defined by the resting condition during sleep. Aliquoted specimens were frozen at −20° C until measurement.

Relaxin was determined by a new radioimmuno assay for human relaxin. The test is based on a rabbit antiserum, raised against recombinant human relaxin-2 (rec-hRLX-2). As tracer ^{125}I-rec-hRLX-2 and as standard material rec-hRLX-2, dissolved in protein containing buffer, was used. The total incubation time was 20-24 hours. To increase sensitivity sequential incubation technique was chosen. In the first step samples and standards were incubated with the antiserum for 16-20 hours and then tracer was added for 3 hours. Under these conditions the following assay characteristics were found: the detection limit, defined as maximum binding minus two standard deviations was 15 pg/ml; the linear range was between 60-2000 pg/ml and the recovery in plasma was 98.5% ± 5.2. Using control sera with 526 pg/ml and 2368 pg/ml we found an intraassay coefficient of variation (CV) of 4.0% and 11.9% (n. = 12). The interassay CVs for the same control sera were 10.7% and 13.1% (n. = 6).

The antiserum showed no cross-reactivity with the following human peptides and proteins: insulin, Zn-insulin, IGF-I, IGF-II, spermolaxin, inhibin-α-subunit, inhibin, seminal-plasma-inhibin-like peptide, CG, LH, FSH, prolactin and ubiquitin.

Total estrogens (estrone and estradiol) excreted into the urine were determined as described ([12]). An aliquot of the urine was incubated for 2 h at 37° C with an enzyme solution (β-glucoronidase/arylsulfatase, Boehringer Mannheim, Germany). The estrogens were then extracted with ether. The aqueous phase was frozen overnight and the organic phase was decanted and evaporated under nitrogen. The concentrations of the estrogens were measured by radioimmunoassay (IBL, Hamburg, Germany). Inter- and intra-assay variations were 8.3% and 5.5% for estradiol and 7.8% and 6.0% for estrone, respectively.

The Wilcoxon-Rank Test was used for statistical analysis.

RESULTS

Basic data, of age, height, weight and time since menopause, of both groups are illustrated in table 1. No significant differences could be seen between the groups. In each treatment group there were 6 cigarette smokers (approx. 20 per day). There were 6 women in the transdermal and 4 in the oral estradiol group who consumed alcohol (approx. 1 pint of beer per day). Thus the clinical data were considered comparable in the two treatment groups.

Table 1. — *Basic data of the estradiol treated patients. Group I received transdermal estradiol, group II oral estradiol (means ± SD).*

	Group I 13 patients	Group II 12 patients
Age (years)	50.1±7.2	53.8±12.1
Height (cm)	165.8±4.9	165.4±5.9
Weight (kg)	66.0±7.9	68.0±10.5
Time since Menopause (years)	4.1±5.5	5.2±4.2
Nicotine use	6 patients	6 patients
Alcohol use	6 patients	4 patients

In all examined patients relaxin could be detected in the urine. Absolute values for the 8 hour excretions before treatment were 6.1 pmol/8 h (SEM 0.5 pmol/8 h) in the transdermal group and 6.2 pmol/8 h (SEM 1.3 pmol/8 h) in the oral group. After 14 days and 28 days of estradiol treatment an increase of 1.3 pmol/8 h and 2.2 pmol/8 h were registered in the transdermal group. In the oral group the increases were 0.9 pmol/8 h and 1.1 pmol/8 h after 14 and 28 days of estradiol treatment, respectively.

A more meaningful expression of the results, however, is given by calculating individually the changes in percentages of the pretreatment values = 100%. The results, calculated in this way, are shown graphically in figure 1. In the transdermal group the relaxin values increased on

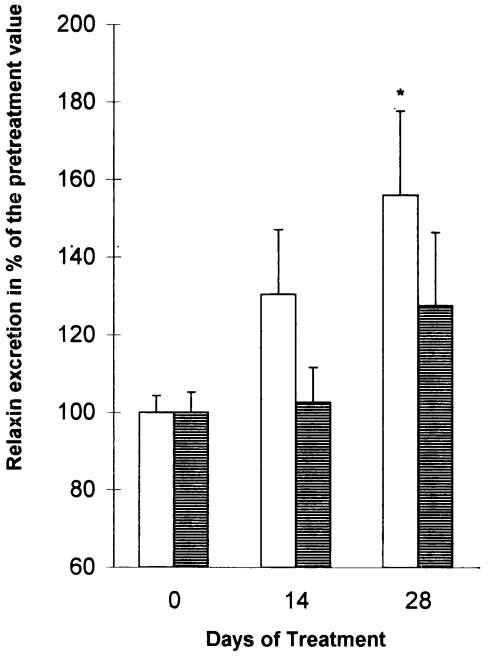

Fig. 1. — Relative excretion of urinary relaxin in postmenopausal women after 14 days and 28 days treatment with transdermal (n. = 13) or oral (n. = 12) estradiol (means ± SEM, * $p < 0.05$). Ordinate: percentage of relaxin excreted in relation to the basal value set as 100%. Abscissa: days of estrogen treatment.
□ Transdermal estradiol group; |≡| Oral estradiol group.

average 30.5% (SEM 16.7%) after 2 weeks and on average 56.1 % (SEM 21.6%) after 4 weeks' treatment. Only the latter result was significantly different to the pretreatment value ($p < 0.05$).

The urinary relaxin excretion in the oral group increased on average 2.6% (SEEM 9.0%) after 2 weeks' and 27.6% (SEM 18.9%) after 4 weeks of treatment. In both instances no statistically significant difference from the pretreatment value could be reached.

The 8 hour excretion of total estrogen in the urine for both groups together with the relaxin/total estrogen-ratio are shown in table 2. As expected, the values of total estrogen were higher in the oral estradiol group than in the transdermal group. The relaxin/estrogen-ratio, however, was significantly ($p < 0.001$) higher after 14 and 28 treatment days in the transdermal than in the oral group.

DISCUSSION

So far no reports on relaxin clearance by the kidneys exists. The present results show that relaxin like insulin ([13, 14]) is excreted in the urine. It may be assumed that urinary relaxin like insulin with which it shares a similar chemical struc-

Table 2. — *Total estrogen excretion and relaxin/estrogen-ratio in the urine of postmenopausal women after transdermal (n. = 13) or oral (n. = 12) estradiol administration before, after 14 days and 28 days of treatment (means ± SEM). Significant difference between transdermal and oral group results = * p < 0.001.*

	Total Estrogen (μ mol/8 h)		
	Before treatment	14 days	28 days
Transdermal Group:	1.2 ± 0.2	3.2 ± 0.3	3.5 ± 0.4
Oral Group:	1.2 ± 0.2	138.8 ± 15.0*	128.1 ± 14.5*
	Relaxin/Estrogen-Ratio ($\times 10^{-6}$)		
	Before treatment	14 days	28 days
Transdermal Group:	5.08 ± 0.60	2.31 ± 0.29	2.37 ± 0.34
Oral Group:	5.20 ± 0.55	0.05 ± 0.006*	0.06 ± 0.004*

ture is related to circulating blood levels under physiological conditions i.e. provided there is normal renal function. The patients examined had no kidney disease, thus urinary relaxin may reflect an increase of systemic relaxin production after transdermal estradiol treatment. However, it cannot be excluded that estradiol treatment has an effect on the kidneys leading to an increased relaxin clearance. A third possibility i.e. the kidneys as a source of the urinary relaxin appears to be rather unlikely.

Johnson *et al.* ([8]) could show that blood levels of relaxin are higher in the second part of the menstrual cycle when estradiol levels are increased compared to the first part. Due to a correlation found only with estradiol but not with gonadotrophins or progesterone the Authors concluded that estradiol may play a certain role in the regulation of the relaxin production. The relaxin blood concentrations measured by Johnson *et al.* were in a similar range to our relaxin concentrations in the urine.

The urinary excretion of total estrogens reflects the different pharmokinetic profiles of the two estradiol administration routes. The excretion after oral estradiol is much higher than after transdermal estradiol. However, due to the first pass effect by the liver which inactivates the major part of estradiol, unmetabolized estradiol reaches the systemic circulation in only a small fraction of the given dose. Transdermal estradiol, escaping first pass degradation, has a much higher bioavailability ([15]).

The relaxin/estrogen-ratio shows that after transdermal treatment low urinary estrogen results in higher relaxin amounts compared to oral administration. In addition, the ratio is constant after 2 and 4 weeks' treatment in the transdermal group compared to an increase after 4 weeks' treatment in the oral group. This may be due to the different blood levels i.e. whereas the estradiol levels are relatively constant after transdermal administration the whole treatment period, concentrations vary considerably after oral treatment showing usually a high peak shortly after administration followed by a quick decrease to base line values ([16]).

The origin of relaxin in postmenopausal women with atrophic genitals is unknown since the usual sources of relaxin, the ovaries and endometrium, are not likely to be the source.

Further investigations are needed to clarify the origin as well as the clearance of relaxin by measuring also blood levels, which was not done in the present study.

Of special interest would be to find out if an estradiol-stimulated relaxin production takes part in the cardioprotective effect of estradiol in postmenopausal women.

REFERENCES

1) Lippert T. H., Struck H., Voelter W. (eds.): "Relaxin - Das wiederentdeckte Hormon". Heidelberg, Germany, Springer Verlag, 1993.
2) Kakouris H., Eddie L. W., Summers R. J.: "Cardiac effects of relaxin in rats". *Lancet*, 1992, *339*, 1076-1078.
3) Han X., Habuchu Y., Giles W. R.: "Relaxin increases heart rate by modulating calcium current in cardiac pacemaker cells". *Circulation Res.*, 1993, *74*, 537-541.
4) Ward D. G., Thomas G. R., Cronin M. J.: "Relaxin increases rat heart rate by a direct action on the cardiac atrium". *Biochem. Biophys. Res. Commun*, 1992, *186*, 999-1005.
5) St. Louis J., Massiotte G.: "Chronic decrease of blood pressure by rat relaxin in spontaneously hypertensive rats". *Life Sciences*, 1985, *37*, 1351-1357.
6) Bigazzi M., Del Mese A., Petrucci F., Casali R., Novelli G. P.: "The local administration of relaxin induces changes in the microcirculation of the rat mesocaecum". *Acta Endocrinol.*, 1986, *112*, 296-299.
7) Bani G., Bani Sacchi T., Bigazzi M., Bianchi S.: "Effects of relaxin on the microvasculature of mouse mammary gland". *Histology and Histopathology*, 1988, *3*, 337-343.
8) Johnson M. R., Carter G., Grint C., Lightmann S. L.: "Relationship between ovarian steroids, gonadotrophins and relaxin during

the menstrual cycle". *Acta Endocrinol.*, 1993, *129*, 121-125.
9) Gangar K. F., Vyas S., Whitehead M., Crook D., Meire H., Campbell S.: "Pulsatility index in internal carotid artery in relation to transdermal oestradiol and time since menopause". *Lancet*, 1991, *338*, 839-842.
10) Mück A. O., Seeger H., Wiesner J., Korte K., Lippert T. H.: "Urinary prostanoids in postmenopausal women after transdermal and oral estrogen therapy". *J. Obstet. Gynecol.*, 1994, *14*, 341-345.
11) Mück A. O., Seeger H., Korte K., Haasis R., Lippert T. H.: "Is there any effect of estradiol on the vasodilating NO/cGMP-system? - investigations of the mechanism of cardiovascular protection by estrogen substitution in postmenopausal women". *Zentralbl. Gynäkologie*, 1994, *116*, 507-511.
12) Bergmeyer H. U., Grassi M., Walter H.-E. In: Bergmeyer H. U. ed.: "Methods of enzymatic analysis". Vol. 2, Weinheim, Germany, VCH, 1983, 206-207.
13) Rabkin R., Ryan M. P., Duckworth W. C.: "The renal metabolism of insulin". *Diabetologica*, 1984, *27*, 351-357.
14) Pickup J. C., Collins A. C. G., Keen H. "Urinary insulin excretion rate: an index of free insulinaemia in insulin-dependent diabetics". *Diabetic Med.*, 1984, *1*, 291-294.
15) Chetkowski R. J., Meldrum D. R., Steingold K. A., Randle D., Lu J., Eggena P. *et al.*: "Biologic effects of transdermal estradiol". *N. Engl. J. Med.*, 1986, *4*, 1615-1620.
16) Powers M. S., Schenkel L., Darley P. E., Good W. R., Balestra J. C., Place V. A.: "Pharmacokinetics and pharmacodynamics of transdermal dosage forms of 17β-estradiol: comparison with conventional oral estrogens used for hormone replacement". *Am. J. Obstet. Gynecol.*, 1985, *152*, 1099-1106.

Address reprint requests to:
Prof. T. H. LIPPERT
Section of Clinical Pharmacology
Department of Obstetrics and Gynecology
Schleichstr. 4
72 076 Tuebingen (Germany)