J. steroid Biochem. Vol. 20, No. 4B, pp. 955–958, 1984
Printed in Great Britain.

0022-4731/84 $3.00 + 0.00

UPTAKE AND METABOLISM OF OESTRIOL IN HUMAN TARGET TISSUES

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Summary—The uptake, metabolism and subcellular distribution of oestradiol and oestriol in endometrial, myometrial and vaginal tissue of postmenopausal women under physiological conditions were studied by giving ^{3}H-labelled oestradiol or oestriol in subphysiological doses by continuous infusion lasting 12 h before hysterectomy. The three tissues obtained from each woman were separated into three fractions: two cytosol fractions (free oestrogens and specifically bound) and one nuclear fraction. The results show an accumulation of both oestrogens in the target tissues, we found an approximately 33 times higher [^{3}H]E_2 concentration in endometrium (dpm per g) than in plasma (dpm/ml), 20 times in myometrium and 10 times in vaginal tissue. After the E_3 infusions the tissue/plasma gradient was 37 for endometrium, 19 for myometrium and 11 for vagina. In plasma and tissues a metabolite of E_3 could tentatively be identified as 16α-hydroxyoestrone. The subcellular distribution showed that 60–80% of E_2 and E_3 is accumulated in the nuclear fraction of all tissues studied, no nuclear bound oestrone could be detected. From these results the conclusion was drawn that oestradiol still is the major tissue oestrogen in postmenopausal women and that it is mainly nuclear bound. Endometrium of postmenopausal women accumulates higher concentrations of E_2 and E_3 than vaginal tissue from the same individual, no preferential uptake of oestriol occurs under physiological conditions.

INTRODUCTION

To exert their biological activities oestrogens have to be taken up by the cells of the so-called target tissues and have to bind to and to activate an intracecullar specific binding protein, the oestrogen receptor. Therefore knowledge of tissue-levels of oestrogens is more relevant to understand the biological effects than the concentration in plasma.

Particularly in postmenopausal women the role of the various oestrogens needs further elucidation. An investigation was initiated to answer the following questions: (1) What is the relationship between the plasma levels of oestradiol and oestriol and the tissue concentration of these oestrogens in endometrium, myometrium and vaginal tissue of postmenopausal women? (2) Does a preferential uptake of any of these oestrogens exist in the three target tissues mentioned? (3) Can differences in uptake, retention and subcellular distribution of oestradiol or oestriol be found between endometrium, myometrium and vaginal tissue?

MATERIAL AND METHODS

Patients

Eleven postmenopausal women, admitted to the Department of Obstetrics and Gynecology to undergo hysterectomy for nononcological reasons, participated after informed consent. More detailed information on these patients and on the techniques used has been published recently [1].

Methods

The patients received a continuous, constant infusion of either [^{3}H]oestrone, oestradiol or oestriol starting 10–12 h before operation. The total activity infused amounted maximally to 60 μCi, total radiation dose was less than 2 mREM. The dose administered equalled less than 5% of the endogenous production of the particular oestrogen during the experimental period, except for oestriol because the production rate of this steroid could not be calculated, the endogenous plasma levels were below the sensitivity limit of our determination at less than 5 pg/ml.

Blood samples were drawn to measure specifically the levels of oestrone, oestradiol and oestriol labelled with ^{3}H. Samples were obtained before anesthesia, 30 min later and at the time of the removal of the uterus. Immediately after extirpation endometrial, myometrial and vaginal tissues were excised by the pathologist. These tissues were processed within 1 h after removal of the uterus.

Tissue processing

Each tissue specimen was separated into five fractions: two cytosol fractions (CF = free oestrogens and CR = oestrogens bound specifically to the oestrogen receptor) and three nuclear fractions (only total nuclear concentrations will be discussed), schematically presented in Fig. 1. To each of the fractions known amounts of nonradioactive oestrogens were added, the steroids were isolated and by reverse

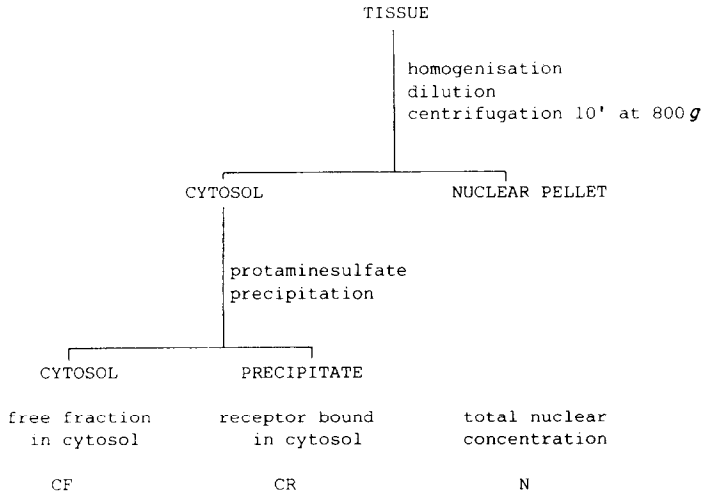

Fig. 1. Schematic outline of the origin of the three subcellular fractions studied.

isotope dilution technique the concentrations of the radioactivity specifically present in each oestrogen could be calculated per g of tissue. Details of the tissue processing have been described recently [1].

The amounts of postmenopausal endometrium and vaginal tissue were too scanty to allow measurements of other parameters, such as oestrogen and progesterone receptors and 17β-hydroxysteroid dehydrogenase activity.

RESULTS

The results of the measurements of radioactive oestrone (E_1), oestradiol (E_2) and oestriol (E_3) in tissue fractions were calculated in dpm/g wet weight, the plasma concentrations in dpm/ml plasma. In order to be able to compare the results of different infusions, all results have been expressed as the ratio of the particular oestrogen in a fraction and its concentration in plasma.

Results obtained after E_2 and E_3 infusions are presented in Table 1. The figures show an accumulation of both oestrogens in the target tissues, the mean tissue concentration of E_2 in endometrium is 33 times higher than in plasma, for myometrium the mean tissue/plasma gradient was 21 and for vagina 10. After E_3 infusions the mean ratio's were: 37 for endometrium, 19 for myometrium and 7 for vaginal tissue. In contrast [^{3}H]E_1 infusions did not lead to an appreciable accumulation in one of the three target tissues, the tissue concentrations of [^{3}H]E_1 were only slightly higher than those in plasma, the mean ratio was less than 2.

Longterm administration of E_1 results in measurable levels of [^{3}H]E_2 due to the peripheral conversion. Calculation after E_1 administration of the mean tissue/plasma ratio for [^{3}H]E_2 showed that this ratio was 15 for endometrium, 9 for myometrium and 11 in vaginal tissue. After E_2 administration, a higher [^{3}H]E_1 gradient was found of 7, 4 and 6 for endometrium, myometrium and vagina respectively.

The results of measurements on the subcellular distribution demonstrated that the high tissue/plasma gradients of E_2 and E_3 were mainly due to their accumulation in the nuclear fractions of all tissues, 60–80% of these oestrogens were found to be present

Table 1. Concentrations of specifically measured oestradiol and oestriol in endometrium, myometrium and vagina of postmenopausal women after continuous infusion of [^{3}H]oestradiol or oestriol resp

Steroid infused		Tissue/Plasma ratio in Endometrium	Myometrium	Vaginal tissue
Oestradiol	CF	2	1	4
	CR	11	5	2
	N	20	15	4
	total	33	21	10
Oestriol	CF	5	1	1
	CR	5	1	1
	N	27	17	4
	total	37	19	7

Tissue concentrations have been expressed as the ratio of the steroid with its level in plasma (=1) at the time of operation. CF = cytosol free fraction, CR = cytosol receptor bound fraction, N = total nuclear fraction, total = sum of the three previous fractions.

in the nuclei of the cells. Again a different pattern was observed for E_1, almost no specifically bound oestrone was present in either the CR or the N fractions. The subcellular distribution of the E_1, originating from peripheral conversions of E_2, was very similar to that after the E_1-infusions, almost no accumulation in the nuclei was seen. E_2 resulting from conversion of E_1 had a subcellular pattern comparable to that after administration of E_2 itself i.e. mainly accumulation in the nuclei of the tissues.

Following the E_1 as well as the E_2 infusions, no metabolites of these oestrogens besides E_2 and E_1 resp. could be detected in either of the tissues studied, more than 90% of the total activity in tissue could be accounted for by these two steroids. Definitely no oestriol was detectable. After the E_3 infusions a metabolite was found behaving comparably to oestradiol during chromatography on Sephadex LH-20 and on gradient elution with Celite columns. Chromatography on thin-layer plates and on paper, before and after acetylation, showed that this metabolite behaved very similarly to 16α-hydroxyoestrone and therefore we believe that the metabolite might be identical to this compound. The presence of this substance was found in plama and in the three tissues studied. The metabolite was present in higher concentrations in tissues than in plasma, its mean tissue/plasma gradient was calculated at 5 in endometrium, 2 in myometrium and 2 in vagina.

DISCUSSION

In our study the uptake, metabolism and subcellular distribution in human endometrium, myometrium and vagina have been examined using subphysiological amounts of radioactive labelled oestrogens administered by continuous infusion to postmenopausal women before hysterectomy. This approach was chosen to enable us to interpret our results in terms of phenomena occurring under physiological conditions.

Our data show interesting differencess between the three oestrogens and between the three tissues studied. Continuous exposure of the tissues to E_2 and E_3 lead to a marked accumulation of radioactivity on the tissues, exposure to E_1 did not result in increased levels of this oestrogen in the tissues. Differences in the ability of the target tissues to concentrate radioactive E_2 and E_3 were clearly seen, the highest concentration always was found in the endometrium, the lowest in the vagina. Therefore the supposed higher sensitivity of vaginal tissue for oestrogenic stimuli cannot be explained by a higher accumulation of oestrogens by that tissue.

The subcellular distribution of E_2 and of E_3 is in contrast with that of E_1, the latter could hardly be found in the receptor bound fraction in cytosol or in the nuclei of the cells. The E_2 accumulation after E_2 but also after E_1 infusions are in agreement with earlier observations on intracellular oestrogens after superfusion of human endometria [2] and with studies on endometria after exogenous stimulation of human postmenopausal women with different oestrogenic preparations [3,4].

The highest intracellular E_1 level was found after E_2 infusions, subcellularly it was present in the free fraction in the cytosol. This is in accordance with the suggestion [1] that part of the intracellular E_1 results from intracellular conversion of E_2, accumulated by the cells. All results point to the fact that in postmenopausal women E_2 still seems to be the major tissue oestrogen. In accordance with our infusion experiments, determinations of the endogenous oestrogens in atrophic postmenopausal endometria demonstrated higher E_2 concentrations per g of tissue than of E_1 despite the higher oestrone plasma levels of these women (C. Vermeulen-Meiners *et al.* submitted for publication). In premenopausal women during the menstrual cycle a similar tissue/plasma gradient for E_2 has been found [5].

The striking similarity in tissue handling of E_2 and E_3 is in accordance with animal experiments [6] showing that these two oestrogens have the same effects on the tissue level when given continuously.

It is remarkable that the atrophic endometrium still is able to concentrate active oestrogens without histological signs of stimulation. An explanation could be the fact that the endogenous concentration of oestradiol in endometria in the proliferative phase of the cycle is the result of a similar gradient between tissue and plasma, indicating that this tissue needs still higher endogenous oestrogen concentrations to show signs of stimulation. Our finding of a metabolite of oestriol, tentatively identified as 16α-hydroxyoestrone, at higher concentrations in tissue than in plasma needs further elucidation, especially in view of the recent hypothesis on the role of the 16α-hydroxylation in breast cancer [7].

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