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EFFECT OF SUPERFUSION WITH HUMAN MALE SERUM, BOVINE SERUM ALBUMIN OR NON RADIOACTIVE ESTROGENS ON THE RETENTION OF TRITIATED ESTRADIOL-17β AND ESTRIOL BY THE RAT UTERUS

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SUMMARY

Slices of mature rat uterus were superfused with either tritiated estradiol-17β or estriol, and subsequently superfused with different washing solutions. The retention of the labeled steroids by the nuclei of glandular and deep stromal cells and by the eosinophils was studied using a dry radioautographic technique for soluble compounds. Superfusion with a washing solution containg non-radioactive estradiol-17β partially displaces either tritiated estradiol-17β or estriol from the nuclei of uterine cells. Superfusion with a washing solution containing either human male serum, bovine serum albumin, or non-radioactive estradiol-17β or estriol partially extracts tritiated estradiol-17β from the uterine eosinophils. Superfusion with a washing solution containing either non-radioactive estradiol-17β or estriol partially displaces tritiated estriol from the uterine eosinophils. Non-radioactive estradiol-17β displaces more label from the nuclei of uterine cells than does non-radioactive estriol, especially when the tracer used is tritiated estriol. On the contrary, non-radioactive estriol displaces more radioactivity from the eosinophils than does non-radioactive estradiol-17β, especially when the tracer is tritiated estradiol-17β. These results suggest that there is some exchange of receptor-bound estrogens with exogenous estrogens for both the cytosol-nuclear and the eosinophil binding systems, and that the cytosol-nuclear and the eosinophil binding systems differ in regard to their retention of estradiol-17β and estriol.

INTRODUCTION

A dry radioautographic analysis of slices of human endometrium, superfused with tritiated estrogens, demonstrated the uptake of radioactivity by eosinophils as well as by the nuclei of epithelial and stromal cells [1]. The uptake by the eosinophils and by the nuclei was similar to that obtained in previous *in vivo* and *in vitro* studies in the rat [2–6]. The labeled compounds were retained by these cells during a prolonged washing with a buffer or bovine serum albumin [1]. A more effective removal of extranuclear steroids was accomplished by washing the labeled slices with human male serum [1], resulting in a radioautographic picture similar to that obtained *in vivo* in the rat [3].

The present study was undertaken in order to compare the retention of tritiated estradiol-17β and estriol by the nuclei of the uterine cells and by the uterine eosinophils under the influence of superfusion with different washing solutions.

* Abbreviations used in this paper: [^{3}H]-E_2 = [2,4,6,7-^{3}H]-estradiol-17β, [^{3}H]-E_3 = [6,7-^{3}H]-estriol, nrE_2 = non-radioactive estradiol-17β, nrE_3 = non-radioactive estriol, KRBG = Krebs–Ringer bicarbonate buffer saturated with 95% O_2–5% CO_2 and containing 1 mg/ml of glucose, HMS = undiluted human male serum, BSA = bovine serum albumin in KRBG.

EXPERIMENTAL

Radioactive steroids

[2,4,6,7-^{3}H]-Estradiol-17β ([^{3}H]-E_2*) (S.A. 90 Ci/mmol) and [6,7-^{3}H]-estriol ([^{3}H]-E_3*) (S.A. 50 Ci/

Table 1. Effect of superfusion with different washing solutions on the retention of radioactivity by the rat uterus previously superfused with tritiated estradiol-17β

Washing solution	Uterine eosinophils	Radioautographic granules in: Nuclei of glandular cells	Nuclei of deep stromal cells	Extracellular space
(−)	32·0 ± 2·4	52·5 ± 2·0	40·5 ± 3·2	18·6 ± 2·1
KRBG	28·2 ± 2·3	56·0 ± 3·6	46·0 ± 3·9	14·3 ± 2·8
BSA	24·8 ± 2·7	56·6 ± 6·4	41·4 ± 3·7	10·2 ± 1·8
HMS	22·4 ± 2·2	53·6 ± 6·2	37·4 ± 4·2	3·9 ± 0·5
nrE_2	15·4 ± 2·8	43·1 ± 4·0	31·5 ± 2·6	11·2 ± 2·5
nrE_3	13·3 ± 1·7	58·8 ± 5·4	42·1 ± 3·6	11·0 ± 3·1

Slices of mature rat uterus were superfused with 20 ng/ml of tritiated estradiol-17β for 60 min, subsequently superfused with different washing solutions for another 60 min and then frozen and submitted to the dry radioautographic technique. Results expressed as average count of radioautographic granules per cell-type ± S.E.M.

mmol) were purchased from New England Nuclear Corp., and purified by paper chromatography.

Superfusion of uterine slices. Rat uteri in the first day of diestrus were sliced at approx. 0·5 mm and placed in a superfusion apparatus described previously [7, 8]. [^{3}H]-E_2 (20 ng/ml) or [^{3}H]-E_3 (3·8 ng/ml), dissolved in a Krebs–Ringer bicarbonate buffer saturated with 95% O_2–5% CO_2 and containing 1 mg/ml of glucose (KRBG*), was superfused over the tissue slices at a rate of 18 ml/h for 60 min at 37°C. At the end of that time, the solution was replaced by a washing medium and the superfusion was continued at the same rate for another 60 min. The washing medium used was either KRBG, undiluted human male serum (HMS*), a solution of bovine serum albumin (BSA*) in KRBG (40 ng/ml), a solution of non-radioactive estriol (nrE_3*) or non-radioactive estradiol-17β (nrE_2*) in KRBG (1 μg/ml).

Samples of tissue were taken at the end of the superfusion with the labeled steroids and at the end of the washing period. The samples of tissue were immediately frozen in liquid propane and stored in liquid nitrogen.

Radioautographic procedure. From each sample, 4 μm cryostat sections were freeze-dried and submitted to the dry radioautographic technique [9]. After 1 or 3 months of exposure, the radioautograms were developed in Kodak developer D-19 at 20°C for 30 sec, then fixed and stained with hematoxylin–eosin or methyl green–pyronine [3].

Quantitative evaluation of the radioautograms. For each experimental condition silver grains in the nuclei of 40 gland cells, in the nuclei of 40 deep stromal cells and in 80 eosinophils were counted. These were chosen at random from eight sections taken from two animals. The radioactivity of the "extracellular space" was estimated by counting 40 areas of a size comparable to an average eosinophil and marked by a circle in the ocular piece of the microscope. These areas were chosen at random between cells located in the deep stroma. In Tables 1 and 2, the results are expressed as the mean for each cell-type ± S.E.M. In Figs. 1 and 2, the results are expressed as % of radioautographic granules per cell-type ± S.E.M. as compared to non-washed uterine slices (taken as 100% for each cell-type).

Table 2. Effect of superfusion with different washing solutions on the retention of radioactivity by the rat uterus superfused with tritiated estriol

Washing solution	Uterine eosinophils	Radioautographic granules in: Nucli of glandular cells	Nuclei of deep stromal cells	Extracellular space
(−)	23·1 ± 1·8	22·5 ± 2·2	18·6 ± 1·2	9·2 ± 1·3
KRBG	21·4 ± 2·0	24·1 ± 1·3	20·5 ± 1·5	6·8 ± 0·7
BSA	21·0 ± 1·6	21·2 ± 1·4	18·9 ± 1·1	6·7 ± 0·6
HMS	21·2 ± 1·9	22·9 ± 1·9	20·5 ± 2·2	4·4 ± 0·5
nrE_2	17·0 ± 1·9	13·3 ± 1·6	10·4 ± 1·0	5·8 ± 0·8
nrE_3	14·3 ± 1·3	18·4 ± 1·4	16·8 ± 1·2	6·6 ± 0·6

Slices of mature rat uterus were superfused with 3·8 ng/ml of tritiated estriol for 60 min, subsequently superfused with different washing solutions for another 60 min and then frozen and submitted to the dry radioautographic technique. Results expressed as average count of radioautographic granules per cell-type ± S.E.M.

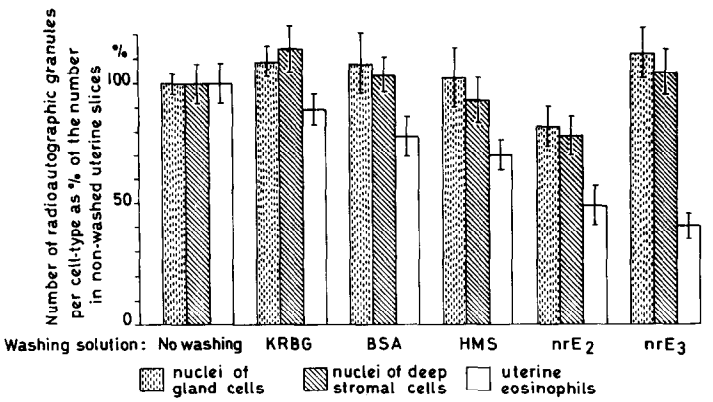

Fig. 1. Effect of superfusion with different washing solutions on the retention of radioactivity but the rat uterus previously superfused with tritiated estradiol-17β. Slices of mature rat uterus were superfused with 20 ng/ml of tritiated estradiol-17β for 60 min, subsequently superfused with different washing solutions for another 60 min and then frozen and submitted to the dry radioautographic technique. Results per cell-type, expressed as % of the number of radioautographic granules in non-washed uterine slices ± S.E.M.

RESULTS

Tables 1 and 2 demonstrate the uptake and retention of radioactive estrogens by the different cell-types of the rat uterus. Upon comparing Table 1 with Table 2, it can be seen that the ratio of uptake of radioactivity by the nuclei of gland or stromal cells to uptake by eosinophils is higher for [^{3}H]-E_2 than for [^{3}H]-E_3.

Table 1 and Fig. 1 represent the effect of superfusion with different washing solutions on the retention of radioactivity by the rat uterus superfused with [^{3}H]-E_2. In the nuclei of uterine cells, only washing with nrE_2 slightly but significantly diminishes the label. For the eosinophils, however, a washing either with HMS or with BSA produces a slight but significant decrease of the label, while nrE_2 or nrE_3 produces a greater decrease. The ratio displacement of [^{3}H]-E_2 from eosinophils to displacement from the nuclei of uterine cells is significantly higher when the washing medium contains nrE_3. In regard to the extracellular space, HMS is the washing medium which extracts the highest amount of radioactivity.

Table 2 and Fig. 2 represent the effect of superfusion with different washing solutions on the retention of radioactivity by the uterus superfused with [^{3}H]-E_3. The nrE_2 solution is the only washing medium which significantly displaces [^{3}H]-E_3 from the nuclei of uter-

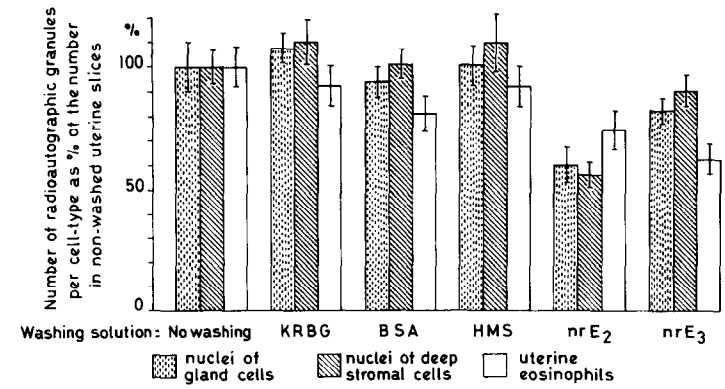

Fig. 2. Effect of superfusion with different washing solutions on the retention of radioactivity by the rat uterus previously superfused with tritiated estriol. Slices of mature rat uterus were superfused with 3·8 ng/ml of tritiated estriol for 60 min, subsequently superfused with different washing solutions for another 60 min and then frozen and submitted to the dry radioautographic technique. Results per cell-type, expressed as % of the number of radioautographic granules in non-washed uterine slices ± S.E.M.

ine cells. For the uterine eosinophils, however, washing with either nrE_2 or nrE_3 produces a significant decrease of the label.

Comparing Fig. 1 with Fig. 2, it is clear that nrE_2 displaces more label from the nuclei of uterine cells than does nrE_3, especially when the tracer used is [^{3}H]-E_3. On the contrary, nrE_3 displaces more radioactivity from the eosinophils than does nrE_2, especially when the tracer is [^{3}H]-E_2.

DISCUSSION

The present investigation demonstrates that the nuclei of the gland and deep stromal cells lose some of the previously bound [^{3}H]-E_2 or [^{3}H]-E_3 when the uterine slices are superfused with a washing solution containing nrE_2. This finding suggests that nrE_2 produces a consumption of the cytoplasmic estrogen receptors, impeding the re-entry of the labeled estrogens into the nuclei once they are released into the cytoplasm. The possibility exists, however, that there is a direct exchange between the tritiated estrogens bound to their receptors in the nuclei and the nrE_2 present in the washing solution.

It is clear from the present results that nrE_2 displaces more label from the nuclei of uterine cells than does nrE_3, especially when the tracer used is [^{3}H]-E_3. This could mean that the 8S or 5S receptors have a lower affinity for estriol than for estradiol-17β, and/or that an easier release of the radioactivity from the nuclei occurs in the case of [^{3}H]-E_3 than in the case of [^{3}H]-E_2. It is interesting to note that washing with HMS, which extracts a significant amount of radioactivity from the extracellular space, does not produce any significant change in the amount of radioactivity in the nuclei.

The uterine eosinophils lose a small but significant amount of [^{3}H]-E_2 after washing with either HMS or BSA. This displacement of radioactivity from eosinophils is higher when non-radioactive estrogens are present in the washing solution, suggesting an exchange of [^{3}H]-E_2 or [^{3}H]-E_3 bound to the eosinophils with the non-radioactive estrogens present in the washing solution.

In agreement with our previous findings of a higher affinity of estriol than of estradiol-17β for the eosinophils [2], the present results show that nrE_3 displaces more radioactivity from the eosinophils than does nrE_2, especially when the tracer is [^{3}H]-E_2. The opposite has been described to occur with the cytosol-nuclear (8S–5S) estrogen binding system, which has a much higher affinity for estradiol-17β than for estriol [2]. These results suggest that the cytosol–nuclear and the eosinophil binding systems differ in regard to their retention of estradiol-17β and estriol.

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