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EFFECT OF HYDROGEN PEROXIDE ON ESTROGEN BINDING BY UTERINE EOSINOPHILS *IN VITRO*

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SUMMARY

The entrance of estrogens into the uterine eosinophils may be responsible for some of the early estrogenic response. The eosinophil peroxidase has been implicated in this theory of action.

This report demonstrates that a low concentration of hydrogen peroxide increases both the uptake and the retention of estrogens by the uterine eosinophils. It is proposed that hydrogen peroxide may act as a terminal hydrogen acceptor in the peroxidase catalyzed estrogen-mediated transfer of hydrogen in the uterine eosinophils.

INTRODUCTION

The eosinophil uptake and retention of estrogens is believed to be responsible for some of the early estrogenic responses in the uterus, such as water imbibition, increase in vascular permeability, histamine releasing and estrogen priming effects [1–6].

The eosinophil peroxidase has been implicated in estrogen action [6]. It had been previously found that hydrogen peroxide significantly increases the amount of tritiated estradiol firmly bound to the uterine eosinophils [7]. It was not clear, however, whether the uptake or the retention of the estrogens was increased. Recently, we have suggested that estrogens bound to the eosinophil peroxidase may play a role as an intermediate hydrogen and electron carrier in a redox cycle in essential oxidative processes [6].

The present study is intended to elucidate further the interaction of estrogens with the eosinophil peroxidase, under the influence of hydrogen peroxide.

EXPERIMENTAL

Materials

[2,4,6,7-^{3}H]-estradiol-17β ($^3HE_2$*) (95 Ci/mmol) from New England Nuclear Corp. was dissolved in ethanol (1 mCi/ml) and diluted in saline.

Methods

Preparation of the uterine tissue. Sprague–Dawley rats in the first day of diestrus were killed by decapitation. The uteri were processed by either of the following procedures: (1) the uterus was sliced into 1 mm thicknesses and the fresh uterine slices were immediately incubated as described below; or (2) the uterus was frozen in liquid propane and 4 μm cryostat sections obtained at -40°C. The cryostat sections were then placed on glass slides, kept at 20°C for 10 min and then incubated as described below.

Incubation of fresh uterine slices. One millimeter uterine slices were incubated at 37°C in a pH 7·4 Krebs–Ringer phosphate glucose buffer containing 5 μCi/ml of $^3HE_2$ and with 0·001 to 0·0001% H_2O_2 in one half of the slices and without H_2O_2 in the other half. After the incubation, the slices were washed in the buffer at 0°C for 20 min, frozen in liquid propane and stored in liquid nitrogen [8]. Four μm cryostat sections were then cut and either water extracted or freeze dried and processed by a dry radioautographic technique [4].

Incubation of the cryostat sections. Cryostat sections of rat uterus were subjected to the following treatment: they were preincubated in saline with or without 20 μg/ml of non-radioactive estradiol-17β (nrE_2*) and with or without 0·001% H_2O_2 [9, 10]. After one of these preincubations, some sections were iodinated in alkaline media [9]. Subsequently, the iodinated and the non-iodinated sections were either extracted with ethanol or washed with saline [9]. Finally, all sections

* Abbreviations used in this paper: $^3HE_2$ = [2,4,6,7-^{3}H]-estradiol-17β; nrE_2 = non-radioactive estradiol-17β.

were incubated with 0·02 μCi/ml of $^3HE_2$ and processed for radioautography [3].

The iodination in alkaline media (Table 2) was intended to destroy the ability of the eosinophil to bind the $^3HE_2$. This destruction occurs unless nrE_2 is "bound" during the iodination [9]. A saturation with nrE_2 during the iodination (Table 2, E and F) gives the maximum protection, so that a subsequent ethanol-extraction of the "protective" estrogens results in a maximum uptake of $^3HE_2$ by the eosinophils [9].

Quantitative evaluation of the radioautograms. For each experimental condition, the number of radioautographic granules in 40 eosinophils were counted in a total of four sections. Twenty of these eosinophils were located in the deep stroma and 20 in the muscular layer, in areas chosen at random [11].

RESULTS

The dry radioautograms of fresh 1 mm uterine slices demonstrated that the presence of H_2O_2 in the incubation medium tremendously increased the uptake of $^3HE_2$ by the uterine eosinophils. Uterine slices incubated in the presence of 0·0001% H_2O_2 showed 62·5 ± 8·9 radioautographic granules per eosinophil when the dry radioautograms were exposed for 6 days (10·42 ± 1·48 radioautographic granules per day of exposure), while slices incubated without H_2O_2 showed 28·4 ± 3·7 radioautographic granules per eosinophil when the radioautograms were exposed for 60 days (0·47 ± 0·06 radioautographic granules per day of exposure). In the water extracted sections, there was a similar increase in the uptake of $^3HE_2$ by the uterine eosinophils caused by the presence of H_2O_2.

Table 1 shows that the uptake of $^3HE_2$ by the uterine eosinophils was greatly decreased in cryostat sections preincubated with nrE_2 either with or without H_2O_2. An ethanol extraction of the nrE_2 previously bound to the uterine eosinophils increased the subsequent binding of $^3HE_2$ only in those sections that had been preincubated with nrE_2. In the sections preincubated with both nrE_2 and H_2O_2 this increase did not occur. The presence of H_2O_2 in a preincubation medium without nrE_2 did not decrease the subsequent uptake of $^3HE_2$ in ethanol-extracted or non-extracted sections.

Table 2 demonstrates a great increase in the uptake of $^3HE_2$ in ethanol-extracted sections after iodination (b), as compared to non-extracted sections after iodination (a), when they were preincubated with nrE_2 without H_2O_2 (E). In sections preincubated with both nrE_2 and H_2O_2, this increase did not occur (F).

DISCUSSION

The dry radioautograms of fresh uterine slices demonstrated that the presence of a low concentration of H_2O_2 in the incubation medium increased the uptake of $^3HE_2$ by the uterine eosinophils. Data from Tables 1 and 2 strongly suggest that the presence of a low concentration of H_2O_2 in the preincubation medium with nrE_2 prevents the subsequent extraction by ethanol of the nrE_2 bound to the eosinophils. These results show that H_2O_2 increases both the uptake and the retention of estrogens by the uterine eosinophils.

It has been previously demonstrated, using an uterine homogenate or a purified peroxidase preparation, that estrogenic steroids were able to mediate the peroxidase-catalyzed transfer of hydrogen between $NADP^+$ and NAD^+ [12, 13] or between the reduced pyridine nucleotides and either hydrogen peroxide [14–16] or another terminal hydrogen acceptor [17, 18]. Since the eosinophil peroxidase constitutes the

Table 1. Effect of non-radioactive estradiol-17β (nrE_2) and H_2O_2 on the uptake of tritiated estradiol-17β ($^3HE_2$) by uterine eosinophils

Preincubation of the uterine sections	Amount of $^3HE_2$ per eosinophil, in sections submitted to the following treatments after preincubation	
	(a) After preincubation, cryostat sections were washed in saline and incubated with $^3HE_2$	(b) After preincubation, cryostat sections were extracted by ethanol and incubated with $^3HE_2$
(A) Saline	30·4 ± 5·6	31·6 ± 5·2
(B) H_2O_2	27·4 ± 3·8	29·1 ± 4·1
(C) nrE_2	4·1 ± 0·6	26·3 ± 4·3
(D) nrE_2 + H_2O_2	1·8 ± 0·3	2·6 ± 0·5

Results expressed as average count of radioautographic granules per eosinophil ± S.E.M. in four experiments for each condition.

Table 2. Effect of non-radioactive estradiol-17β (nrE_2) and H_2O_2, followed by iodination, on the uptake of tritiated estradiol-17β ($^3HE_2$) by uterine eosinophils

Preincubation of the uterine sections before iodination	Amount of $^3HE_2$ per eosinophil, in sections submitted to the following treatments after iodination: (a) After iodination, cryostat sections were washed in saline and incubated with $^3HE_2$	(b) After iodination, cryostat sections were extracted by ethanol and incubated with $^3HE_2$
(A) Saline	5·2 ± 0·7	8·0 ± 0·9
(B) H_2O_2	4·6 ± 0·6	4·4 ± 0·6
(C) Saline, followed by extraction by ethanol	5·0 ± 0·6	5·6 ± 0·9
(D) H_2O_2, followed by extraction by ethanol	3·4 ± 0·5	3·2 ± 0·5
(E) nrE_2	4·7 ± 0·8	22·8 ± 4·4
(F) nrE_2 + H_2O_2	2·7 ± 0·4	2·8 ± 0·5
(G) nrE_2, followed by extraction by ethanol	4·1 ± 0·6	5·6 ± 1·0
(H) nrE_2 + H_2O_2, followed by extraction by ethanol	2·4 ± 0·5	3·1 ± 0·6

Results expressed as average count of radioautographic granules per eosinophil ± S.E.M. in four experiments, for each condition.

only source of peroxidase in the uterus (see [6] for a review), it is possible that the eosinophil peroxidase is the enzyme involved in the above reaction. Furthermore, the present experiments suggest that hydrogen peroxide may act as a terminal hydrogen acceptor in the peroxidase-catalyzed estrogen-mediated transfer of hydrogen in uterine eosinophils.

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