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IODINATION OF ESTROGEN RECEPTORS IN RAT UTERINE EOSINOPHILS

A. TCHERNITCHIN, X. TCHERNITCHIN and A. M. BONGIOVANNI
The Children's Hospital of Philadelphia, Department of Pediatrics, The University of Pennsylvania School of Medicine, 1740 Bainbridge Street, Philadelphia, Pa. 19146, U.S.A. and Department of Biology and Genetics, University of Chile Medical School, Zañartu 1042, Santiago, Chile

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SUMMARY

Iodination of cryostat sections of rat uterus in an alkaline media destroys the ability of the eosinophil receptors to bind tritiated estradiol-17β, as demonstrated by radioautography. Non-radioactive estradiol-17β bound to its receptors in uterine eosinophils during the iodination protects the receptors from the destruction by iodination, suggesting that iodination interacts with the active site of the estrogen receptor in eosinophils. This protection by exogenous or endogenous estrogens against the destruction by iodination of the eosinophil receptors provides a direct method for assay of endogenous estrogens bound to their receptors in eosinophils as well as a method for assay of estrogens in biological fluids.

INTRODUCTION

Two important different estrogen binding systems have been found to exist in the rat uterus: the 8S–5S and the eosinophil binding systems [1–3].

The 8S–5S binding system [4–6] consists of a cytoplasmic receptor in the 105,000*g* supernatant–the 8S receptor [7, 8] and a nuclear 5S receptor [9, 10]. This system is thought to be responsible for the genomic response, that is, the RNA and protein synthesis and the true growth of the uterus [2, 11].

The eosinophil binding system has been demonstrated *in vitro* [12–15] and *in vivo* [3] in the uterus of the mature rat. Uterine eosinophils are considered to be involved in some of the early estrogenic response in the uterus, that is, the water imbibition, histamine releasing and estrogen priming effects [2, 15].

Several biochemical studies have been done to characterize the 8S and the 5S estrogen receptors (see [11] for a review). Puca and Bresciani [16] demonstrated that iodination of the 8S and 5S calf uterine receptors in an alkaline media destroys their ability to bind estradiol-17β, and that the iodination is ineffective when the hormone is interacting with the binding site, thus protecting it from a direct effect.

The present investigation is intended to demonstrate that the eosinophil receptors share the characteristic of the 8S and 5S receptors described by Puca and Bresciani [16] and to devise a new histochemical method for the assay of endogenous estrogens bound to uterine eosinophils.

EXPERIMENTAL

Materials

Preparation of the uterine tissue. Sprague–Dawley rats in proestrus, estrus and in the first day of diestrus were killed by decapitation. The uteri were immediately frozen in liquid propane and 4μ cryostat sections were obtained at

−40°C. The fresh frozen sections were placed on glass slides and kept at 20°C for 10 min before the experiment.

(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 to obtain a final steroid concentration of 0·02 μCi/ml.

Methods

Ethanol extraction. Uterine sections mounted in glass were immersed for 10 min in 100% ethanol at 20°C for the extraction of the estrogens bound to uterine eosinophils. This procedure has been found to partially extract the estradiol-17β from the uterine eosinophil receptors without altering their capacity for estrogen binding[13]. Control sections were washed in saline for a similar period of time instead of being extracted with ethanol. The extraction with ethanol was performed at different stages of the experimental procedure: either before or after the incubation with non-radioactive estradiol-17β (nrE_2*), and/or after the iodination, etc. as stated under "experimental procedure".

Incubation with nrE_2. Each uterine section was incubated for 10 min at 20°C in 0·2 ml of solution containing 20 μg/ml of nrE_2 in saline. All sections incubated with nrE_2 were thoroughly washed in saline immediately after the incubation and before any subsequent experimental procedure.

Iodination. Each uterine section submitted to iodination was incubated for 20 min in 0·2 ml of a 0·037 M I_2 and 0·214 M KI solution in Tris-K^+-EDTA medium (pH 8·5)[16]. The iodination was performed at 20°C. At the end of the iodination, the reaction was stopped by adding 0·6 ml of a 0·2 M $Na_2S_2O_3$ solution[16]. The sections were thoroughly washed in saline before any subsequent procedure.

Incubation with $^3HE_2$. Uterine sections were incubated for 10 min at 20°C with 0·2 ml of a solution containing 0·02 μCi/ml of $^3HE_2$ (50 pg/ml of 10 pg of $^3HE_2$ per uterine section). After the incubation, the sections were thoroughly washed in running water and dried at 20°C for subsequent radioautography.

Radioautographic method. The sections were dipped in radioautographic emulsion Kodak NTB-3 melted at 38°C. The slides were exposed for 8 days and developed in D-19 at 20°C for 30 sec[2]

Quantitative evaluation of the radioautograms. For each experimental situation the number of radioautographic granules in 40 eosinophils were counted in a total of 4 sections. 20 of these eosinophils were located in the deep stroma and 20 in the muscular layer, in areas chosen at random[13]. The results in Tables 1 and 3 are expressed as % of radioautographic granules per eosinophil ± S.D.M. as compared to the controls in column d (non-iodinated sections that have been extracted with ethanol prior to the incubation with $^3HE_2$), which represents the total of the eosinophil receptors.

Experimental procedure

Experiment N° 1: Effect of iodination on the uptake of $^3HE_2$. (see Fig. 1). Ethanol-extracted or non-extracted sections of rat uterus in diestrus were submitted to iodination (the experimentals) or incubated in saline (the controls). Subsequently, the sections were incubated with $^3HE_2$ and then processed for radioautography.

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

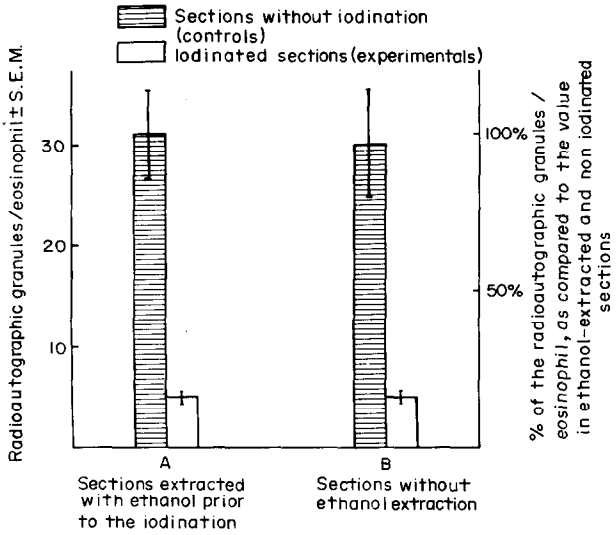

Fig. 1. Effect of iodination on the uptake of tritiated estradiol-17β by the uterine eosinophils of rats in diestrus (see "experimental", exp. N°1).

Average count of radioautographic granules ± S.D.M. is expressed in absolute number of silver grains per eosinophil and as % of the count as compared to ethanol-extracted and non-iodinated sections.

Experiment N° 2: Effect of iodination on the $^3HE_2$ *previously bound by uterine eosinophils.* Cryostat sections of rat uterus in diestrus were incubated with $^3HE_2$, subsequently they were submitted to iodination (the experimentals) or incubated in saline (the controls), and then processed for radioautography.

Experiment N° 3: Effect of nrE_2 *and iodination on the uptake of* $^3HE_2$. (see Table 1). Cryostat sections of rat uterus in diestrus were submitted to one of the following pretreatments: (A) extraction with ethanol, (B) washing in saline in-

Table 1. Effect of non-radioactive estradiol-17β (nrE_2) and iodination on the uptake of tritiated estradiol-17β ($^3HE_2$) by uterine eosinophils (see "experimental", Exp. No. 3). Results expressed as % of the radioautographic granules per eosinophil ± S.D.M. as compared to the controls in column d

Pre-treatment of the uterine sections before the iodination (*)	Amount of $^3HE_2$ per eosinophil, in sections submitted to the following treatments: a	b	c	d
	(1) Iodination (2) Saline (3) $^3HE_2$	Iodination Ethanol $^3HE_2$	Control (saline) Saline $^3HE_2$	Control (saline) Ethanol $^3HE_2$
(A) Ethanol	16·0% ± 2·0	18·0% ± 3·2	101·2% ± 15·8	100% ± 14·0
(B) Saline	16·4% ± 2·1	25·3% ± 3·0	96·2% ± 17·8	100% ± 16·5
(C) nrE_2, followed by Ethanol	15·8% ± 2·4	21·4% ± 4·0	102·5% ± 18·7	100% ± 16·1
(D) nrE_2	18·0% ± 3·4	84·2% ± 16·5	20·4% ± 4·1	100% ± 18·0
(E) Ethanol, followed by nrE_2	19·7% ± 3·5	82·1% ± 12·9	19·7% ± 3·2	100% ± 15·7

*The different pre-treatments before the iodination were intended to have the eosinophil binding sites free of estrogen (A, B and C) or saturated with nrE_2 (D and E) during the iodination.

stead of the extraction with ethanol, (C) incubation with nrE_2, followed by extraction with ethanol, (D) incubation with nrE_2, or (E) extraction with ethanol, followed by incubation with nrE_2. After one of these pretreatments, the sections were iodinated (a and b, the experimentals) or incubated in saline (c and d, the controls). After this procedure, the sections were extracted with ethanol (b and d) or washed in saline (a and c). Subsequently all sections were incubated with $^3HE_2$ and processed for radioautography.

Experiment N° 4: Effect of the hormonal condition of the rat on the uptake of $^3HE_2$ after iodination and/or ethanol-extraction of endogenous estrogens (see Table 3). Cryostat sections of rat uterus in proestrus, estrus and in the first day of diestrus, were submitted to either extraction with ethanol or washing in saline. Afterwards, the sections were iodinated (a and b, the experimentals) or incubated in saline (c and d, the controls). After this procedure, the sections were extracted with ethanol (b and d) or washed in saline (a and c). Subsequently all sections were incubated with $^3HE_2$ and processed for radioautography.

RESULTS

1. *Effect of iodination on the uptake of $^3HE_2$*

The iodination of the cryostat sections of the rat uterus in the first day of diestrus reduces significantly the uptake of $^3HE_2$ by uterine eosinophils ($P < 0{\cdot}01$) (Fig. 1). In animals in the first day of diestrus the decrease of uptake of $^3HE_2$ following iodination is similar in the uterine sections that were or were not extracted with ethanol prior to the iodination (Fig. 1).

2. *Effect of iodination on the $^3HE_2$ previously bound by uterine eosinophils*

The iodination of the uterine sections, when effectuated after the incubation of these sections with $^3HE_2$, does not extract any appreciable amount of $^3HE_2$ that has been bound to uterine eosinophils prior to the iodination. The iodinated sections show $29{\cdot}7 \pm 4{\cdot}2$ silver grains per eosinophil and the control sections show $30{\cdot}4 \pm 5{\cdot}5$ silver grains per eosinophil, essentially identical.

3. *Effect of nrE_2 and iodination on the uptake of $^3HE_2$*

Table 1 shows the effect of nrE_2 and iodination on the uptake of $^3HE_2$ by uterine eosinophils. The different pretreatments before the iodination were intended to have the eosinophil binding sites free of estrogen (A, B and C) or saturated with nrE_2 (D and E) during the iodination. The extraction with ethanol after the iodination (column b), as compared to non-extracted sections (column a) produced a significant increase in the uptake of $^3HE_2$ in sections that have had their eosinophil-receptor sites saturated during the iodination (D and E)($P < 0{\cdot}01$) but not in sections with the eosinophil-receptor sites free of estrogen during the iodination (A, B and C). A similar increase in the uptake of $^3HE_2$ occurs in non-iodinated sections that have been ethanol-extracted prior to the incubation with $^3HE_2$ (d, as compared to c) only when the eosinophil receptor sites were saturated with nrE_2 (A, B and C)($P < 0{\cdot}01$). These results are summarized in Table 2.

4. *Effect of the hormonal condition of the rat on the uptake of $^3HE_2$ after iodination and/or ethanol-extraction of endogenous estrogens*

Table 3 summarizes the effect of the hormonal condition of the rat on the up-

Table 2. Effect of non-radioactive estradiol-17β (nrE_2) and iodination on the uptake of tritiated estradiol-17β ($^3HE_2$) by uterine eosinophils. Summary of results in Table 1

Amount of nrE_2 bound to the uterine eosinophils during the iodination	Amount of $^3HE_2$ in eosinophils, in sections submitted to the following treatments			
	a	b	c	d
	(1) Iodination (2) Saline (3) $^3HE_2$	Iodination Ethanol $^3HE_2$	Control (saline) Saline $^3HE_2$	Control (saline) Ethanol $^3HE_2$
low	+	+	+++	+++
high	+	+++	+	+++

+++ High labeling in eosinophils.
+ Low labeling in eosinophils.

Table 3. Effect of the hormonal condition of the rat on the uptake of tritiated estradiol-17β ($^3HE_2$) by uterine eosinophils after iodination and/or ethanol-extraction of endogenous estrogens (see "experimental", exp. No. 4). Results expressed as % of radioautographic granules per eosinophil ± S.D.M. as compared to controls in column d

Pre-treatment of the sections prior to the iodination	Hormonal condition of the rat	Amount of $^3HE_2$ per eosinophil in sections submitted to the following treatments:			
		a	b	c	d
		(1) Iodination (2) Saline (3) $^3HE_2$	Iodination Ethanol $^3HE_2$	Control (saline) Saline $^3HE_2$	Control (saline) Ethanol $^3HE_2$
Saline	Proestrus	12·2% ± 3·1	80·4% ± 14·7	17·9% ± 4·1	100% ± 16·2
Saline	Estrus	11·6% ± 2·8	71·3% ± 12·8	26·6% ± 5·1	100% ± 14·1
Saline	First day of diestrus	16·4% ± 2·1	25·3% ± 3·0	96·2% ± 17·8	100% ± 16·5
Ethanol	Proestrus	13·1% ± 2·0	14·1% ± 2·9	94·7% ± 19·1	100% ± 15·2
Ethanol	Estrus	17·6% ± 4·2	19·7% ± 3·5	92·1% ± 14·2	100% ± 18·0
Ethanol	First day of diestrus	16·0% ± 2·0	18·0% ± 3·2	101·2% ± 15·8	100% ± 14·8

take of $^3HE_2$ by uterine eosinophils after the iodination. The extraction with ethanol after the iodination (column b, as compared to column a) produces a significant increase in the uptake of $^3HE_2$ in rats in proestrus or in estrus ($P < 0{\cdot}01$), but not in the rats in the first day of diestrus. This increase parallels the increase in the uptake of $^3HE_2$ produced by ethanol-extraction in non-iodinated sections (d, as compared to c). These differences between rats in the first day of diestrus and those in proestrus or in estrus disappear when the uterine sections are ethanol-extracted prior to the iodination. In the ethanol-extracted sections prior to the iodination, the results in rats under all hormonal conditions resemble those of rats in the first day of diestrus that have not been ethanol-extracted before the iodination.

DISCUSSION

The present report demonstrates that iodination of cryostat sections of rat uterus in an alkaline media destroys the ability of the eosinophil receptors to bind

estradiol-17β. This property has already been described for the 8S and the 5S calf uterine receptors[16].

Ethanol has been demonstrated to extract an important amount of estradiol-17β from uterine eosinophils without altering their capacity for estrogen binding (13). This property, and a lack of extraction of estradiol-17β from the eosinophil-receptors by iodination (as demonstrated by the experiment No. 2), provides a method for the study of the effect of estradiol on the iodination. Experiment No. 3 demonstrates that the estradiol bound to the uterine eosinophil receptors during the iodination protects the receptors against the destruction by iodination, suggesting that the iodination interacts with the active site of the estrogen receptor in eosinophils. An extraction with ethanol of this "protective" estradiol from the eosinophil receptors after the iodination permits a subsequent uptake of $^3HE_2$ by uterine eosinophils in direct proportion to the amount of nrE_2 bound to them during the iodination. In the case of the eosinophil receptors saturated with nrE_2 during the iodination, the protection is maximum and subsequent extraction of the "protective" estrogens from the receptors results in a maximum uptake of $^3HE_2$ by uterine eosinophils. The opposite occurs when the eosinophil receptors are free of estrogen during the iodination and thus exposed to destruction.

The protection by endogenous or exogenous estrogens against the destruction by iodination of the eosinophil receptors provides a direct method for assay of endogenous estrogens bound to their receptors in eosinophils, as well as a method that could be used for an assay of "exogenous" estrogens from biological fluids that could be bound to uterine eosinophils prior to the iodination. As an example of assay of "endogenous" estrogens, experiment No. 4 demonstrates in rats under different hormonal conditions that the amount of $^3HE_2$ bound to the eosinophils in ethanol-extracted sections after the iodination is proportional to the endogenous estrogens bound to their receptors during the iodination.

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REFERENCES

1. Tchernitchin A., Hasbún J., Peña G. and Vega S.: *Proc. Soc. exp. Biol. Med.* **137** (1971) 108.
2. Tchernitchin A.: *Steroids* **19** (1972) 575.
3. Tchernitchin A. and Chandross R.: *J. steroid Biochem.* **4** (1973) 41.
4. Shyamala G. and Gorski J.: *J. cell Biol.* **35** (1967) 125A.
5. Jensen E. V., Suzuki T., Kawashima T., Stumpf W. E., Jungblut P. W. and DeSombre E. R.: *Proc. Natn. Acad. Sci. U.S.A.* **59** (1968) 632.
6. Shyamala G. and Gorski J.: *J. biol. Chem.* **244** (1969) 1097.
7. Toft D. and Gorski J.: *Proc. Natn. Acad. Sci. U.S.A.* **55** (1966) 1574.
8. Rochefort H. and Baulieu E. E.: *C. R. Acad. Sci. (Paris)* **267** (Serie D) (1968) 662.
9. Jensen E. V., DeSombre E. R., Hurst D. J., Kawashima T. and Jungblut P. W.: *Arch. Anat. Microsc. Morphol. exp.* **56** (Suppl.) (1967) 547.
10. Puca G. A. and Bresciani F.: *Nature* **218** (1968) 967.
11. Jensen E. V. and DeSombre E. R.: *Ann. Rev. Biochem.* **41** (1972) 203
12. Tchernitchin A.: *Steroids* **10** (1967) 661.
13. Tchernitchin A.: *Steroids* **15** (1970) 799.
14. Tchernitchin A., Chandross R. and Glasser R. L.: *Fed. Proc.* **31** (1972) 258.
15. Tchernitchin A.: *5th Int. Congr. Pharmacol.*, San Francisco, volunteer abstracts (1972) p. 231.
16. Puca G. A. and Bresciani F.: *Nature* **225** (1970) 1251.