J. steroid Biochem. Vol. 36, No. 4, pp. 301–303, 1990
Printed in Great Britain.

0022-4731/90 $3.00 + 0.00

UTERINE ESTROGEN SULFATASE ACTIVITY. INFLUENCE OF STEROID HORMONES AND ADENINE NUCLEOTIDES

MARIÁ CRISTINA LOZA,* ANTONIO VALENCIA[1] and JUAN JOSÉ HICKS
Division of Biology of Reproduction and [1]Molecular Biology Biomedial Research Unit, Mexican Institute of Social Security, Mexico

(*Received* 8 *November* 1989)

Summary—Steroid sulfatase enzymes participate greatly in reproductive events. To date, estrogen sulfatase seems to have a regulatory role in the control of free estrogen levels in target tissues.

The present study evaluates the participation of some adenine nucleotides in estrogen sulfatase kinetics. Using ADP, ATP, NAD and the combination of ADP + NAD or ATP + NAD it was found that adding either of the combined cofactors, the enzymatic activity increased more than 2.0 times.

In ovariectomized rats, the corresponding mean enzyme activity was found to be higher than in intact rats. It was also found, in ovariectomized rats treated with ovarian hormones, an inhibition that was higher with estradiol-17β than with progesterone treatment.

This data suggests that the estrogen sulfatase, being a hormone-dependent enzyme, participates in a new control mechanism of estrogen levels in presence of some cofactors and free steroids.

INTRODUCTION

The participation of steroid sulfates in several metabolic processes has previously been demonstrated. Steroid sulfates are intermediates and storage forms in steroid biosynthesis and metabolism in endocrine and hepatic tissues [1, 2]. In fertile women, the levels of estrone sulfate (E_1-S) are higher than those estradiol-17β (E_2), as much as 10–20-fold [3, 4]. This estrogen conjugate can be partially hydrolyzed by the estrogen sulfatase, present in several mammalian tissues [1, 5, 6] including placenta [7] and fetal tissues [8], into estrone (E_1) and then transformed into E_2 by the 17β-hydroxysteroid oxidoreductase in the presence of reduced cofactors (NADH and NADPH) [9].

The ubiquitous distribution of steroid sulfatase in target tissues, particularly endometrium [10], accompanied by high levels of E_1S in circulation [3], suggests that this enzyme could have a regulatory effect on free estrogen levels. The regulatory role of the enzyme may have some clinical significance in postmenopausal women with low peripherical E_2 levels, or in those women with breast cancer correlated with high levels of E_1S [11, 12].

The cofactors requirement for the estrogen sulfatase activity has not been established as yet. In previous reports [13,14], we found that the rat adrenal steroid sulfatase enhanced its activity by the addition of ADP, ATP and NAD; c-AMP did not present any effect on this system. The purpose of the present study was to evaluate the *in vivo* and *in vitro* participation of some factors associated with the estrogen sulfatase activity in different hormonal conditions.

*To whom correspondence should be addressed: Dr María Cristina Loza, Division of Biology of Reproduction, Biomedical Research Units, IMSS, P.O. Box 12-855, México, D. F. México.

EXPERIMENTAL

Chemicals

[6,7-^{3}H]Estrone sulfate, 52.5 Ci/mmol, was purchased from New England Nuclear Co. and its radiochemical purity checked by thin-layer silica gel chromatography in the system toluene:dioxane:acetic acid (20:10:1), prior to use. Unlabeled conjugates, free steroids NAD, ADP and ATP were purchased from Sigma Chemical Co.

Animals and treatment

Adult Long–Evans rats (200 g average weight) with regular estrous cycles (having two regular cycles by daily vaginal smears) were divided into two groups of 30 animals each.

Groups A and B received different treatments. Group A was killed after presenting diestrous, and uteri were immediately obtained and cleaned. Homogenization was done in Krebs–bicarbonate buffer, pH 7.2, containing 30 mM nicotinamide, in such a way that 0.3 ml contained 10 mg of uterine

tissue. Aliquots of 0.3 ml of homogenate were incubated for 2 h at 37°C with 300 dpms of [6,7-^{3}H]E_1S, adjusting the mass to 40 nmol by the addition of cold E_1S in presence of either 3 mM NAD, 1 mM ADP or 1.6 mM ATP and in the following combinations NAD + ADP or NAD + ATP, in the same concentrations as when used separately. The incubations were carried out without the addition of exogenous TPNH. In order to avoid further transformation of E_1 to more polar estrogens. Blanks without enzyme or with boiled enzyme were included with each batch.

The rats in group B were bilaterally ovariectomized. Twenty days after surgery a number of them were used as a control (treated only with vehicle), the rest of the group was treated for four days with several doses of E_2 or progesterone (P), dissolved in propylene glycol (0.5, 5 and 10 μg/day of E_2 and 5 and 10 mg/day of P) by intramuscular administration. On the fifth day, animals were sacrificed. Uterine horns were prepared in the same way that group A's were. The incubation was carried out in presence of 3 mM NAD and 1.6 mM ATP. Protein concentration was determined by Lowry's method [15].

Extraction

At the end of the incubation period, the estrone liberated was extracted by a simple partition between the aqueous medium and the organic solvent and then quantified. In a previous experiment, the following solvent systems were used as the organic solvent: benzene: ether/chloroform (4:1), and scintillating solvent (50 mg POPOP 4 g PPO dissolved in 1 l toluene) [16, 17]. Practically all the unconverted estrone sulfate remained in the aqueous phase.

RESULTS

Table 1, shows the recovery of [^{3}H]E_1 that was extracted by each of the solvent systems. The scintillating solvent extracted 97.2% of the estrone formed and only 4.3% of the untransformed precursor.

The comparative degree of desulfation of E_1S by uterine estrogen sulfatase in the presence of some adenine nucleotides is illustrated in Table 2. The addition of ADP, ATP or NAD increased the specific activity of the enzyme in 1.16, 1.32 and 1.80 times respectively, compared with that of the controls.

The addition of NAD combined with ADP or ATP increased the hydrolitic activity of the sulfatase 2.19 and 2.61 times respectively. The results in Table 3, show the behavior of the rat uterine estrogen sulfatase activity in different conditions. In metestrous (estrous cycle phase with low estrogen levels), the E_1 sulfatase was found to be 10.99 nmol/mg protein/h.

In control group B, twenty days after surgery, the hydrolysis of E_1S by the uterine tissue was 1.77 times higher than that of rats during metestrous.

One can see in Table 3, that E_2 inhibited the hydrolysis of E_1S in a dose-dependent response, the respective values were 28.6, 49 and 61.2% (0.5, 5 and 10 μg of 17β-E_2 injected/day) compared to the levels of the ovariectomized group without any treatment (control). In uteri of ovariectomized rats treated with 5 and 10 mg/day of P, the inhibition of the enzymatic activity obtained was 17.6 and 20.7% respectively, compared to the activity found in untreated animals.

Table 1. [6,7-^{3}H]Estrone and [6,7-^{3}H]estrone sulfate recoveries from Krebs bicarbonate buffer, pH 7.2 by the organic solvent systems

Solvent	[^{3}H]E_1[a]	[^{3}H]E_1S[a]
Benzene	100 ± 7.25	4.7 ± 0.7
Ether/cloroform	100 ± 6.35	9.7 ± 1.2
Liquid scintillation[b]	97.2 ± 6.82	4.3 ± 0.8

[a]The amount of radioactive estrogens (300 dpm equivalent in 40 nmol) dissolved in 0.1 ml ethanol were placed on the bottom of the incubating tubes and 2.0 ml of buffer and 20 ml of the organic solvent were added to carry out the partition.
[b]50 mg POPOP and 4 g PPO dissolved in 1 l toluene.
Values are means of 6 individual assays and are given in % ± SD.

Table 2. *In vitro* effect of adenine nucleotides on estrogen sulfatase activity of rat uterus at diestrous

Cofactor added (mM)	[^{3}H]E_1 released	Ratio cofactor control
Control	4.95 ± 0.48	
NAD 3.0	8.91 ± 1.00	1.80
ADP 1.0	5.73 ± 0.43	1.16
ADP 1.0 + NAD 3.0	10.86 ± 1.21	2.19
ATP 1.6	6.55 ± 0.67	1.32
ATP 1.6 + NAD 3.0	12.93 ± 0.89	2.61

Incubations were carried out in triplicates. Results expressed as nmol/mg protein/h ± SD, are means of 6 individual assays.

Table 3. *In vivo* efferct of steroid hormones on uterine estrogen sulfatase activity of 20 days ovariectomized rats

Group B conditions	Daily injected doses	[^{3}H]E_1 released (nmol/mg protein/h)	±SD	No. of experiments
Metestrous	—	10.99	0.65	10
Ovx control[b]	—	19.50	0.98	12
Ovx + E_2	0.5 μg	13.92	1.02	9
	5.0 μg	9.95	0.71	16
	10.0 μg	7.57	0.36	14
Ovx + P	5.0 mg	16.08	0.58	15
	10.0 mg	15.85	1.00	17

[a]Values are means for the number of experiments enlisted in the right column.
[b]20 days ovariectomized rats without any treatment.

DISCUSSION

The relations between conjugated/unconjugated steroids associated with biological events such as reproductive cycles [18], sperm capacitation [19] gestation and some diseases, has brought about new interest to the biological role and regulation of the sulfotransferase and sulfatase activities, which appear to be under some biological control.

In previous studies, we have shown that the highest enzymatic activity was found in the mitochondrial fraction; when steroid sulfatase was tested using [^{3}H]pregnenolone sulfate as a substrate, being significantly increased in the presence of Ca^{2+}. On the other

hand, the enzymatic activity was diminished with high Mg^{2+} concentrations [20]. It is interesting to point out in relation to the cofactor requirements, that a stimulatory effect upon the uterine estrogen sulfatase activity due to the combination of ATP + NAD, showed an increase of 2.6 times. Similar results have been reported for rat adrenal steroid sulfatase [14]. It is possible that the sulfate ions released in the enzymatic reaction join ATP, and form the 3-phosphoadenylyl sulfate (APS) [21], thus causing a sulfate concentration deficiency in the system. Consequently, the enzymatic reaction is displaced favoring the formation of E_1. Another hypothesis is that the allosteric site in the enzyme recognizes the ATP molecule producing its activation. The increment obtained when using ADP may be produced by the equilibrium established by the adenylate cyclase action.

The addition of NAD to the incubation medium increased the enzymatic activity by 80%. This nucleotide may also act as an allosteric activator such as ATP. Naturally these hypothesis require further investigation.

Ovariectomized rats in group B treated with 0.5, 5 or 10 μg/day of E_2 for four days, showed an inhibition of 28 and 61% respectively in the enzymatic activity. These results in a dose–response way, suggest that perhaps E_2 could be acting as a modulator of the uterine estrogen sulfatase activity by a negative feedback mechanism. The pattern did not occur in ovariectomized rats treated with P where the inhibition was not in a dose–response manner; the effect was similar with both doses tested and, it was less than that observed with E_2 (Table 3). Based on the results reported here and according to previous communications [13, 14, 22], we suggest that estrogen sulfatase of the rat uterus is a hormone-dependent enzyme which may play a role in the regulatory mechanism of free estrogen levels. Finally, we propose that some adenine nucleotides and ovarian hormones are related to the enzymatic activity control.

Acknowledgement—Part of these reults were presented as a thesis work by María Teresa Ruiz for a degree in Biology, Facultad de Ciencias, Universidad Nacional Autónoma de México, México (1983).

REFERENCES

1. Pack B. A. and Brooks S. C.: Metabolism of estrogen and their sulfates in the rat uterine minces. *Endocrinology* **87** (1970) 924–933.
2. Adessi G. L., Shuan T. Q. and Vingier P.: *In vivo* and *in vitro* metabolism of estrone and estradiol-17β and their 3-sulfates in pregnant female guinea pigs; a plausible prehormone role of estrogen sulfates in the maternal uterus *J. Steroid Biochem.* **16** (1982) 107–116.
3. Brown B. and Smith B. J.: Oestrone sulfate the major circulating oestrogen in the normal cycle. *J. Reprod. Fert.* **24** (1972) 142–149.
4. Purdy R. H., Engel L. L. and Oncley J. L.: The characterization of estrone sulfate from human plasma. *J. Biol. Chem.* **236** (1961) 1043–1049.
5. Gurpide E., Gusberg S. B. and Tseng L.: Estradiol binding and metabolism in human endometrial hyperplasia and adenocarcinoma. *J. Steroid Biochem.* **7** (1976) 891–896.
6. Warren J. C. and French A. P.: Distribution of steroid sulfatase in human tissues. *J. Clin. Endocr. Metab.* **25** (1965) 278–282.
7. Tran Quang N., Guy-Martin M. E. and Adessi G. L.: Role des ovaires maternels et des visoeres foetaux dans la synthese et le metabolism des oestrogenes produits par la placenta de cobaye *in vitro*. *Ann. Biol. Anim. Biochim. Biophys.* **19** (1979) 1737–1744.
8. Platia N. P., Elking-Hirsch K. E., Fenci M. and Tulchinsky D.: Possible biological importance of estrone sulfate in the human. *The Endocrine Soc. Abstracts of Papers, 64th Annual Meeting*, San Francisco, Calif. (1982) p. 243.
9. Tseng L., Gusberg S. B. and Gurpide E.: Estradiol receptor and 17β-dehydrogenase in normal and abnormal human endometrium. *Ann. N.Y. Acad. Sci.* **286** (1977) 190–198.
10. Carlstrom K., von Vexkull A. K., Einhorn N., Fredricsson B., Lunell N. O. and Sundelin P.: Metabolism of estrone sulfate in human endometrium. *Acta Obstet. Gynec. Scand.* **62** (1983) 519–524.
11. Jassonni V., Bulleti C., Franceschetti F., Bunavia M., Bolelli G., Ciotti P. and Flamigni C.: Estrone sulphate plasma levels in postmenopausal women with and without endometrial cancer. *Cancer* **53** (1984) 2698–2700.
12. Wilking N., Carlstrom K., Gustafsson S. A., Skodefors H. and Tollbom O.: Oestrogen receptors and metabolism of oestrone sulphate in human mammary carcinoma. *Eur. J. Cancer* **16** (1980) 1339–1346.
13. Domínguez O. V., Loza A. C., Morán L. Z. and Valencia S. A.: ACTH and sulfatase activity. *J. Steroid Biochem.* **5** (1974) 867–875.
14. Domínguez O. V., Valencia S. A. and Loza A. C.: On the role of steroid sulfates in hormone biosynthesis. *J. Steroid Biochem.* **6** (1975) 301–309.
15. Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J.: Protein measurement with the Folin-phenol reagent. *J. Biol. Chem.* **193** (1951) 265–275.
16. Burstein S. and Dorfman R. I.: Determination of mammalian steroid sulfatase with 7-^{3}H-3β-hydroxy-androst-5-en-17-one-sulfate. *J. Biol. Chem.* **238** (1963) 1656–1660.
17. Domínguez O. V., Loza C. A., Guadarrama E. and Valencia A. S.: Interactions between pregnenolone-sulfate and DHEA-sulfate as substrates of the rat adrenal steroid sulfatase and the effect of "*in vivo*" stimulation with ACTH. *The Endocrine Soc. Abstracts of Papers. 55th Annual Meeting*, Chicago, Ill. (1973) p. 164.
18. Dwyer R. J. and Robertson H. A.: Oestrogen sulphatase and sulphotransferase activities in the endometrium of the sow and ewe during pregnancy. *J. Reprod. Fert.* **60** (1980) 187–191.
19. Legault Y., Bouthillier M., Bleau G., Chapdelaine A. and Roberts K. D.: The sterol and sterol sulfate content of the hamster reproductive tract. *Biol. Reprod.* **20** (1979) 1213–1219.
20. Gilson-Jose O., Domínguez O. V. and Valencia S. A.: Pregnelonona sulfatase da membrana mitocondrial do testiculo de rato. *Ciencia Cultura* **27** (1975) 642–645.
21. Roy A. B.: Enzymological aspects of steroid conjugation. In *Chemical and Biologial Aspects of Steroid Conjugation* (Edited by S. Burstein and S. Solomon). Academic Press, New York (1970) pp. 74–130.
22. Loza A. C. and Hicks J. J.: Inhibición de la estrogeno sulfatasa uterina por estrógenos libres. *Rev. Iberoam. Fert. Reprod. Hum.* **VI** (1989) 129–133.