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Oviduct 17β-Estradiol Receptor in the Female Lizard, *Podarcis s. sicula*, during the Sexual Cycle: Relation to Plasma 17β-Estradiol Concentration and Its Binding Proteins

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ABSTRACT—In the oviparous lizard, *Podarcis s. sicula*, 17β-estradiol binding molecules were characterized in the oviduct and plasma. In addition, their concentration was evaluated throughout the annual cycle.

In the oviduct, a 17β-estradiol receptor (ER) is present. It shows high affinity for ligand ($4.9–7.0 \times 10^{-10}$ M). It is decreased by ovariectomy, and induced by 17β-estradiol treatment. 17β-estradiol causes also a ER shift from the cytosol into the nuclei. On sucrose gradient ER behaviour is consistent with the properties of ER obtained from other 17β-estradiol target organs. At isoelectrofocusing the labeled 17β-estradiol binding molecules fall into three pH ranges: 5.2–5.6, 7.0–7.7, 8,0–8.7.

Nuclear filled ER significantly increases as oviduct grows. This supports an ER role in oviduct stimulation by 17β-estradiol.

In the plasma, 17β-estradiol binding molecules show many physico-chemical and behavioural properties of steroid-binding proteins (SBPs).

INTRODUCTION

In the lizard, *Podarcis s. sicula*, like in most oviparous reptiles living in temperate zones, the female genital apparatus is active during spring and keeps quiescent for the rest of the year [1]. In early spring, as animals leave their winter shelters, several ovarian follicles undergo vitellogenesis, becoming yolky and ripe in a few weeks. Ovarian resumption is accompanied by parallel progressive growth of the oviduct, which rapidly reaches maturity. Reproductive females usually lay one to three egg clutches (three to six eggs per clutch at 20-day intervals), starting from the first half of May. In late spring, the breeding season ceases, the ovary becomes quiescent, and the oviduct regresses assuming a typical thread-shaped aspect [2, 3].

The mature oviduct shows complex gross morphology and includes four regions, cranio-caudally: infundibulum, tuba, uterus and vagina. Histologically, it is characterized by considerable hypertrophy of the epithelium and associated glands. Moreover, oviduct development and activity give rise to several biochemical events involving RNA and protein synthesis [4, 5], as well as some hydrolytic enzyme activity [4]. Lizard eggs are supplied with little albumen and a coriaceous shell, which are secreted by tubal and uterine glands, respectively [6, 2].

Seasonal oviduct growth depends on ovarian sex hormones, since, in prereproductive females, it can be easily prevented by ovariectomy and restored by 17β-estradiol or testosterone injections into spayed females [7]. Moreover the presence of sex hormone-binding molecules in oviduct cytosol and nuclei, has been also reported [8].

This work was undertaken with the aim of defining the properties of oviduct 17β-estradiol binding molecules in lizards, and ascertaining their putative receptor nature. Moreover, the concentration of these molecules has been evaluated

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throughout the breeding period and in 17β-estradiol and/or progesterone treated and spayed females, in relation to the plasma titres of steroid binding proteins (SBPs) and sex hormones.

MATERIALS AND METHODS

Animals Adult females of *Podarcis s. sicula* were caught in the outskirts of Naples during October 1990–July 1991. Soon after capture, the animals were anaesthetized with ethyl ether and bled through a heparinized glass capillary inserted in the heart. After brief centrifugation, the plasma was stored in liquid nitrogen until use. At autopsy, the oviducts were rapidly removed, rinsed in 0.7% saline solution in order to eliminate any blood trace, weighed and plunged in liquid nitrogen. Macroscopical oviduct development and ovarian follicle diameters were assessed by direct inspection, and the tissues were divided into four groups, according to the stage of oviduct development:

1. Quiescent stage (October–February). Oviducts showed a thread-like aspect (about 19 mg weight each). The ovary contained only previtellogenetic follicles.

2. Recovery stage (March–April). Oviducts were growing and each of them weighed about 30 mg. In the ovary, several follicles were engaged in vitellogenetic processes.

3. Full growth stage (April–June). Oviducts appeared full grown, and each of them weighed about 80 mg. In each ovary 1 to 3 ripe follicles were ready to ovulate.

4. Secretive stage (June–July). Eggs were contained in the oviduct which weighed about 60 mg.

For experimental studies lizards, caught both in October and June, were reared in terraria at a temperature of 28°C, with a photoperiod L : D = 16 : 8, and fed on meal worms and vegetables *ad libitum*. October lizards were ovariectomized; four weeks later they were divided into four groups, each of 20 animals, and treated for two weeks as follows.

Group 1: injected every two days intraperitoneally with 0.1 μg of 17β-estradiol (Sigma) dissolved in 0.1 ml of 0.7% saline.

Group 2: injected with 0.1 μg of progesterone (Sigma) dissolved as before.

Group 3: injected with both 0.1 μg of 17β-estradiol and 0.1 μg of progesterone.

Group 4: injected with 0.1 ml of solvent.

Twenty-four hours after the last injection, the lizards were killed and processed as reported before.

June intact lizards with secretive oviducts, were divided into two groups, each of 10 animals. Group 1 animals were injected intraperitoneally with 0.1 μg of 17β-estradiol (Sigma) dissolved in 0.1 ml of 0.7% saline. Group 2 animals received the solvent alone. The lizards were killed six and twenty-four hours later, and their oviducts were utilized as reported above.

Preparation of oviduct subcellular fractions All procedures were carried out at 0–4°C. Analytical grade chemicals were used. Oviducts were weighed, minced and homogenized in 3.5 vol (w/v) of TEMG (10 mM Tris-HCl, 1 mM EDTA, 1 mM 2-mercaptoethanol, 10% glycerol, pH 7.8), containing 0.05 M NaCl (homogenization buffer). The suspension was centrifuged at 800×*g* for 10 min. The supernatant was centrifuged at 105,000 ×*g* for 1 hour in order to obtain the cytosol. The 800×*g* pellet was suspended in 10 vol of buffer Tris-HCl 10 mM containing 3 mM $MgCl_2$, 2 mM monothioglycerol, 0.25 M sucrose, pH 7.5 (rinsing buffer), pelletted and rinsed twice with the same buffer volume. Final pellet was suspended in 3.5 vol. (w/v) of TEMG containing 0.7 M KCl (extraction buffer). This suspension was frozen, thawed, and left 1 hour in ice bath with occasional stirring. The suspension was thereafter centrifuged at 105,000×*g* for 1 hour. The supernatant constituted the nuclear extract.

Measurement of ^{3}H-17β-estradiol binding in cytosol and nuclear extract (2,4,6,7-^{3}H)-17β-estradiol (90/110 Ci/mmole) was purchased from Amersham Radiochemical Centre (Amersham, Bucks, U.K.); unlabeled steroids were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Endogenous steroids were previously stripped from samples by adding an equal volume of TEMG containing 0.05% (w/v) dextran (Dextran T-70, Pharmacia Fine Chemicals, Piscataway, NJ USA) and 0.5% (w/v) active charcoal (Norit A

charcoal-Sigma, St. Louis, MO USA). The mixture was vortexed and incubated for 10 min at 4°C. The charcoal was removed by centrifugation at 3000 rpm for 10 min. The steroid-free supernatants were utilized for all subsequent analyses.

Aliquots (0.2 ml) of cytosol and nuclear extract were incubated with 5 nM labeled 17β-estradiol, with or without a 100-fold excess of diethylstylbestrol (DES) (DES does not bind to steroid binding proteins in plasma, therefore it is used to discriminate between 17β-estradiol binding sites in plasma and in tissues). After 16 hours incubation at 4°C, 0.6 ml of active charcoal suspension was added to the incubation medium. The mixture was vortexed and kept for 5 min in ice bath; thereafter it was centrifuged at $800 \times g$ for 10 min at 4°C. The supernatant was decanted in counting vials and added with Maxifluor scintillation fluid (Maxifluor, Packard, Milan. Italy). Radioactivity was measured in a Liquid Scintillation Spectrometer (Packard 1600-CA) at 45% counting efficiency.

For K_d determinations, 0.2 ml aliquots of cytosol and nuclear extracts were added to tubes containing increasing amounts (0.3 to 5 nM) of labeled 17β-estradiol, with or without a 100-fold excess of DES. Incubations were carried out for 16 hours at 4°C. Bound and unbound steroids were separated by adding 0.6 ml of charcoal-dextran suspension as reported before. Specific binding data were analyzed according to Scatchard's graphic method [9].

Labeled 17β-estradiol unfilled (RU) binding sites were determined by incubation 0.2 ml of samples with 5 nM labeled 17β-estradiol with or without a 100-fold excess of DES for 30 min at 0°C. Total binding sites (RT) were determined by incubating similar mixtures for 30 min at 20°C. The filled binding sites (RF) were calculated by the formula: RT − RU = RF [10].

For binding specificity evaluation, 0.2 ml samples were added to 5 nM of labeled 17β-estradiol with or without increasing amount (10^{-9}–10^{-6} M) of various unlabeled steroids. Incubation and separation of bound and unbound steroids were performed as reported before.

Measurement of ^{3}H-17β-estradiol binding in the plasma Endogenous steroids were removed as reported above. For K_d determination, plasma aliquots (0.2 ml), diluted with TEMG to a protein concentration of 2 mg/ml, were used. Proteins were determined by Lowry *et al.*'s (1951) method [11], using BSA as a standard. Samples were added to tubes containing increasing amounts (0.3 to 20 nM) of labeled 17β-estradiol, with or without a 100-fold excess of unlabeled 17β-estradiol. Tubes were incubated at 0°C for 1 hour and thereafter added with 0.6 ml of dextran-charcoal suspension. The mixtures were briefly vortexed and incubated 1 min at 0°C. After centrifugation, supernatants were decanted in counting vials and added with 5 ml Maxifluor scintillation fluid to evaluate radioactivity as reported before. Binding specificity was determined by incubating samples (0.2 ml) with 20 nM of labeled 17β-estradiol with increasing amounts (10^{-9}–10^{-6} M) of various unlabeled steroids. Incubation procedures and radioactivity evaluation were as reported before.

Isoelectrofocusing The method reported by Matsumada and Goldman was used [12]. Cytosolic and nuclear extract samples (0.2 ml) were preincubated with 5 nM labeled 17β-estradiol for 1 hour at 4°C; plasma samples were subjected to the same incubation procedure although with 20 nM labeled 17β-estradiol. A glass column (31×0.5 cm) was filled with a mixture of 12.5% sucrose in water containing 0.01% Triton X-100 and 3% Ampholine (Pharmacia, Sweden) pH 3.5–10. After a prerun of 1 hour at 4°C, 200 V, the samples were layered on top of the gradient. Electrofocusing was carried out for 16 hours at 4°C, 200 V. At the end of the run, 0.4 ml aliquots were removed from the bottom of the column and used for the evaluation of the pH gradient and 17β-estradiol binding as described above.

Sucrose density gradient Aliquots (0.2 ml) of cytosol, nuclear extract and plasma were preincubated with labeled 17β-estradiol (5 nM for tissue extracts and 20 nM for plasma) for 1 hour at 4°C. Afterwards they were layered on the top of 4.4 ml of 5–20% sucrose linear gradient in TEMG containing 0.7 M KCl, and centrifuged at $189{,}000 \times g$ at 4°C for 16 hours. Ovalbumin (3.7 S), albumin (4.6 S) and catalase (10.5 S) (Sigma) were run as

markers in parallel gradients. After centrifugation, the gradient were fractionated by collecting 0.2 ml aliquots from the punctured bottom of the tubes. Samples were reincubated with 5 nM labeled 17β-estradiol with or without a 100-fold excess of DES or 17β-estradiol, in order to evaluate the specific binding.

Measurement of plasma 17β-estradiol and progesterone A radioimmunoassay (RIA) method adapted to *Podarcis s. sicula* plasma was employed [13]. Sensitivity was 3 pg for both 17β-estradiol (intraassay variability 7%; interassay variability 13%) and progesterone (intraassay variability 6%; interassay variability 9%).

Statistical analysis Numerical data were analyzed by the ANOVA method, followed by the Duncan multirange test.

RESULTS

Labeled 17β-estradiol binding molecules were present both in the cytosol and the nuclei of the lizard oviduct, and showed a high ligand affinity ($K_d = 7.0 \times 10^{-10}$ M for cytosol and $K_d = 4.9 \times 10^{-10}$ M for nuclear extract) (Fig. 1A, B). Labeled 17β-estradiol binding molecules were found also in the plasma, though showing lesser ligand affinity ($K_d = 0.8 \times 10^{-8}$ M) (Fig. 1C). The affinity values did not undergo significant modifications in the various phases of the breeding cycle (Table 1).

Fig. 2 reports the specificity of labeled 17β-estradiol molecules in cytosol, nuclear extract and plasma samples. Both 17β-estradiol and DES competed very well in the nuclear extract, though specificity curves suggest that labeled 17β-estradiol molecules bind 17β-estradiol thighter than DES, while corticosterone, progesterone and testosterone competed poorly. 17β-estradiol competed very well in the cytosol, followed by progesterone, testosterone, DES and corticosterone. In the plasma, progesterone, testosterone and corticosterone competed with labeled 17β-estradiol molecules, whereas DES was inefficacious.

Table 2 reports the levels of oviduct unfilled (RU) and filled (RF) labeled 17β-estradiol binding

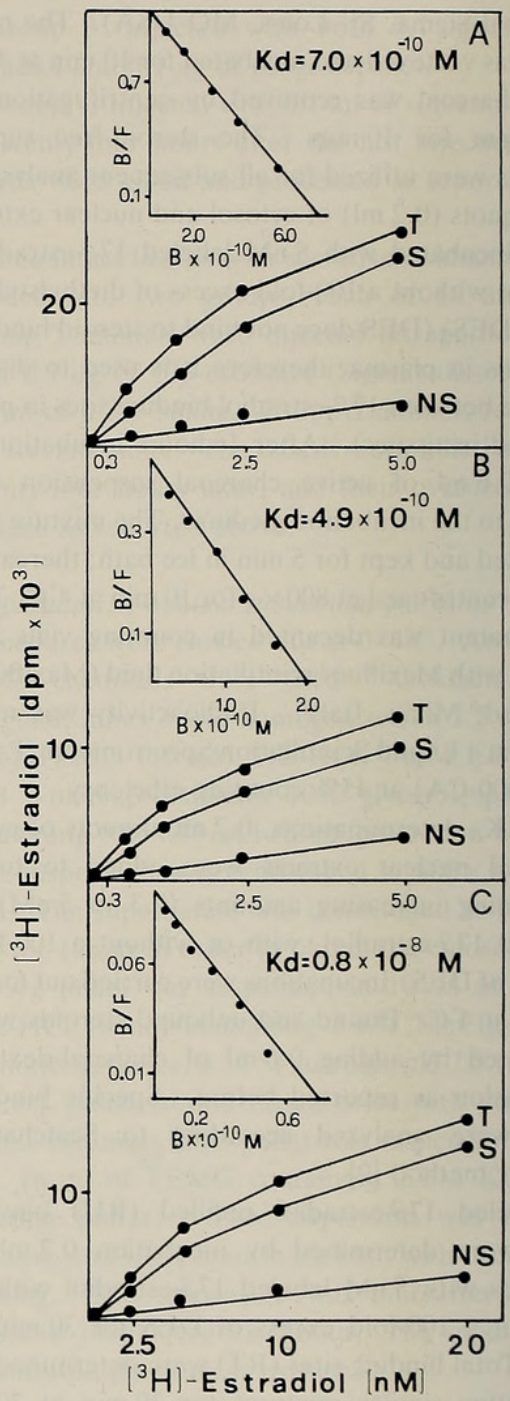

Fig. 1. Saturation curve and Scatchard analysis of 17β-estradiol binding molecules in the oviduct cytosol (A) and nuclear extract (B) and in the plasma (C) of lizard, *Podarcis s. sicula* during different phases of the reproductive period. (A) cytosol of quiescent oviduct; (B) nuclear extract of secretive oviduct; (C) plasma (February). T=Tolal; S=Specific NS= Not specific.

sites during the breeding cycle. In the cytosol and nuclear extract both RU and RF binding sites were always present; however, RU sites constantly prevailed. RU reached the highest level in quiescent

TABLE 1. K_d of the ^{3}H-17β-estradiol binding activity in the lizard *Podarcis s. sicula* oviduct and plasma during the reproductive cycle

Oviduct stage	(n)*	Cytosol	Nuclear extract	Plasma
Quiescent	15	$7.0\pm0.9\times10^{-10}$ M	$12.1\pm3.4\times10^{-10}$ M	$0.8\pm0.3\times10^{-8}$ M
Full-grown	8	n.d.**	$1.3\pm0.2\times10^{-10}$ M	$1.5\pm0.4\times10^{-8}$ M
Secretive	8	$8.9\pm1.1\times10^{-10}$ M	$4.7\pm0.5\times10^{-10}$ M	$0.1\pm0.1\times10^{-8}$ M

Each value is the mean $\pm$ SE of three different determinations.
* number of animals for each determination.
** not calculated because abnormal scatchard curue profiles.

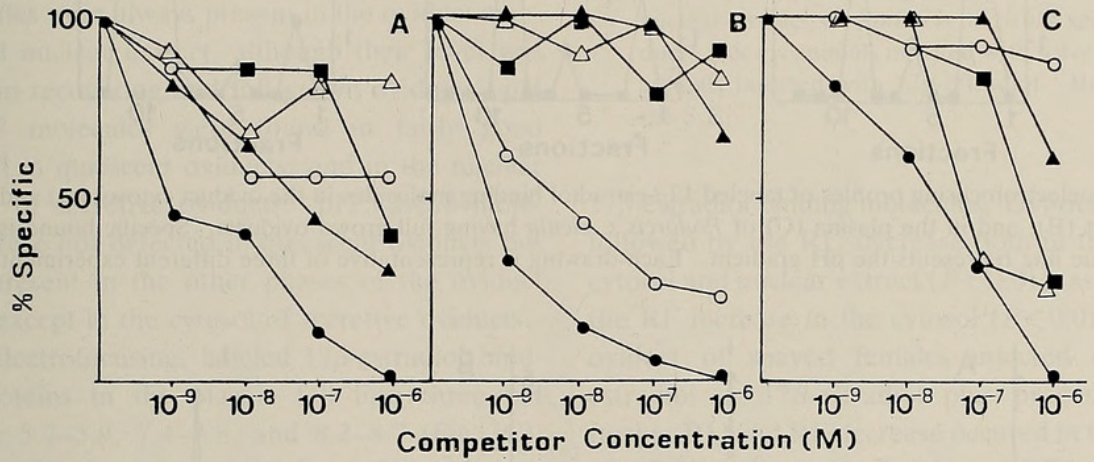

FIG. 2. Labeled 17β-estradiol binding specificity in the oviduct cytosol (A) and nuclear extract (B), and in the plasma (C). Samples refers to *Podarcis s. sicula* having full grown oviducts. Incubation were carried out with labeled 17β-estradiol and increasing amounts of competitors (10^{-9}–10^{-6} M). Competition is expressed as a percentage of specific labeled 17β-estradiol binding. (△=Corticosterone; ○=Diethylstylbestrol; ■=Testosterone; ▲=Progesterone; ●=17β-estradiol).

TABLE 2. Changes in the level of oviduct estradiol binding activity, plasma estradiol binding activity and plasma estradiol and progesterone in the lizard, *Podarcis s. sicula* during the reproductive cycle

Oviduct stages	(n)*	Oviduct estradiol binding activity (fmol/g tissue)				Plasma estradiol binding activity (fmol/mg protein)	Plasma concentration	
		Cytosol		Nuclear extract			Progesterone (ng/ml)	Estradiol (ng/ml)
		Receptor unfilled#	Receptor filled#	Receptor unfilled#	Receptor filled#			
Quiescent	6	13108±1543	991±141	4711±568	371±35	86.4± 9.3	4.5±1.1	1.2±0.3
Recovery	5	3577± 365	405± 49	3266±319	643±65	n.d.**	n.d.**	n.d.**
Full-growth	2	220± 35	145± 11	2408±223	452±31	145.7±15.1	60.0±9.9	1.4±0.4
Secretive	3	1156± 131	164± 13	717± 68	173±15	71.8± 6.7	4.5±1.1	0.2±0.06

Each value is the mean $\pm$ SE of four different determinations.
* number of animals per each determination.
** not dosed.
# Receptor filled=17β-estradiol binding sites occupied by the endogenous hormone at the time of the assay. Receptor unfilled=17β-estradiol binding sites not occupied by the endogenous hormone at the time of the assay.

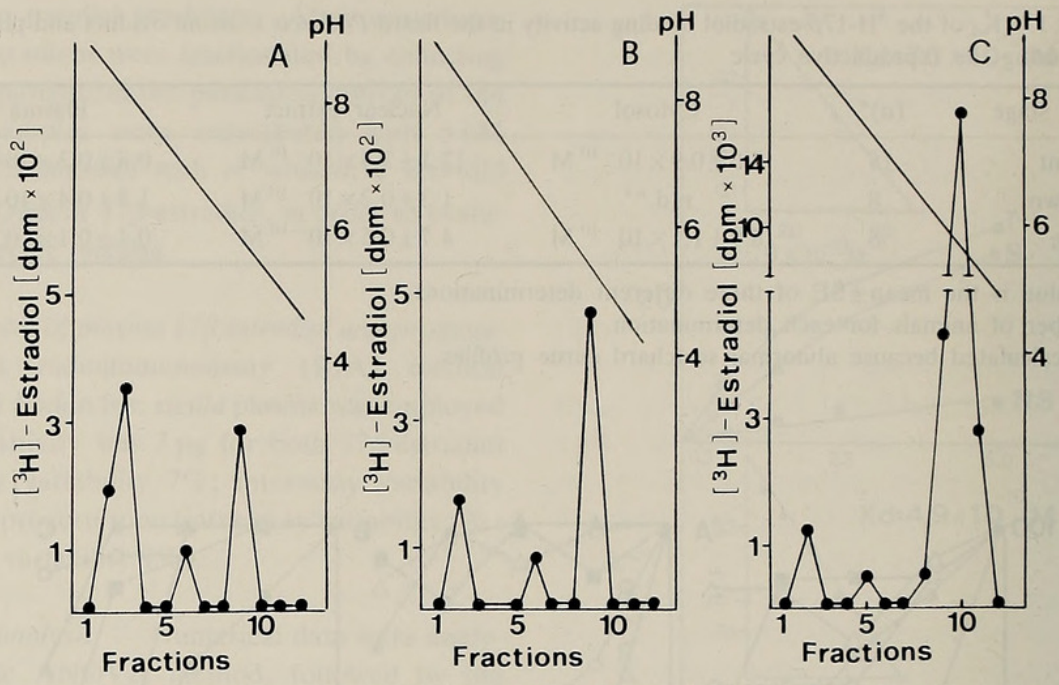

Fig. 3. Isoelectrofocusing profiles of labeled 17β-estradiol binding molecules in the oviduct cytosol (A) and nuclear extract (B), and in the plasma (C) of *Podarcis s. sicula* having full grown oviducts. Specific bound is shown. Oblique line represents the pH gradient. Each drawing is representative of three different experiments.

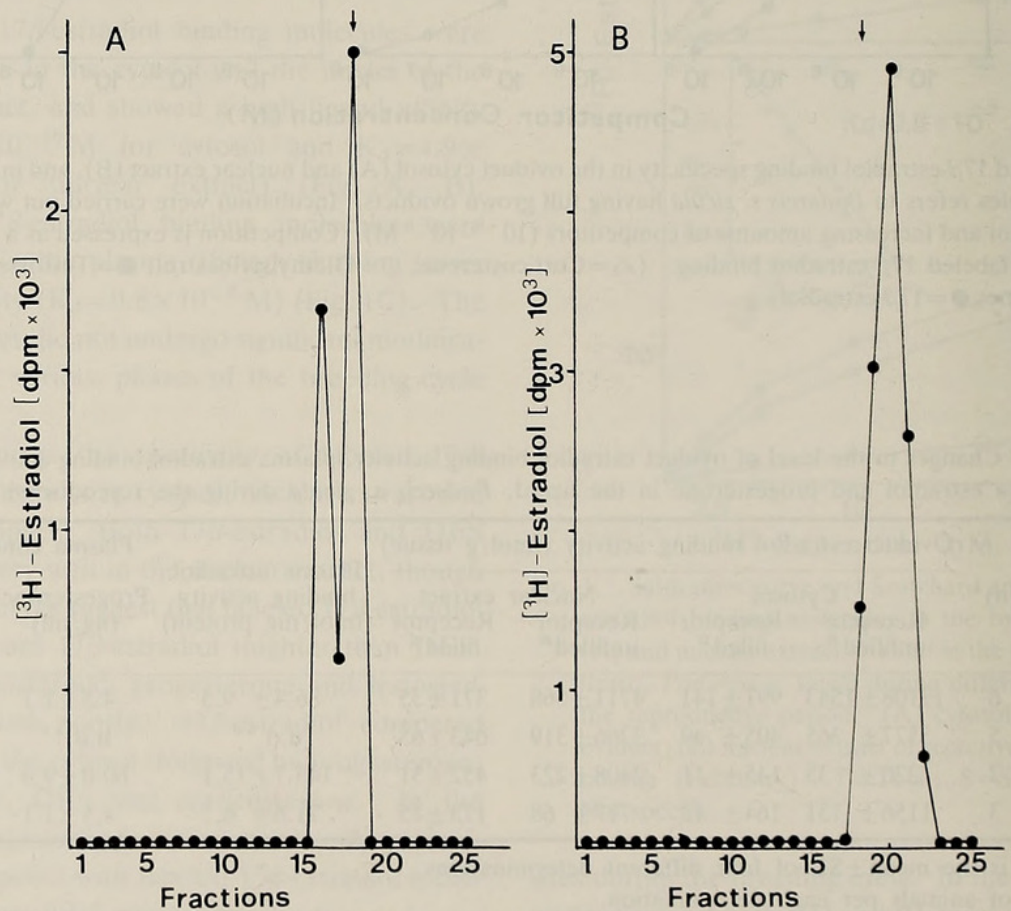

Fig. 4. Sucrose gradient profile of labeled 17β-estradiol molecules in the nuclear extract (A) and in the plasma (B) of *Podarcis s. sicula* having full grown oviducts. Only the specific binding is shown. Each drawing is representative of three different experiments. The arrow indicates the Albumin (4.6 S) position along the sucrose gradient.

oviducts and decreased as oviduct growth occured during the breeding period. RF sites showed a similar behaviour in the cytosol, but they were significantly higher in nuclear extracts of recovering and full grown oviducts.

The labeled 17β-estradiol binding activity in the plasma increased during ovarian vitellogenesis; the same happened for plasma levels of progesterone and 17β-estradiol (Table 2).

At electrofocusing, the labeled 17β-estradiol binding molecules from both oviduct cytosol and nuclear extract fell into three pH ranges: 5.2–5.6, 7.0–7.7, and 8.0–8.7 (Fig. 3A, B). pH 8.0–8.7 molecules were always present in the oviduct cytosol and nuclear extract, although their level was lower in recovering and full grown oviducts. pH 7.0–7.7 molecules were found in fairly good amount in quiescent oviducts, and in the nuclear extract of secretive oviducts. pH 5.2–5.6 molecules were not detected in quiescent oviducts but were present in the other phases of the oviduct cycle, except in the cytosol of secretive oviducts.

At electrofocusing, labeled 17β-estradiol binding proteins in the plasma fell into three pH ranges: 5.7–5.9, 7.4–7.8, and 8.2–8.7 (Fig. 3C). These molecules were always detectable, although pH 5.7–5.9 molecules were the most abundant, and significantly increased during oviduct growth.

On sucrose gradient, labeled 17β-estradiol binding molecules of the oviduct cytosol sedimented at about 4.6 S, regardless of the oviduct stage (not shown). Nuclear extract binding molecules, however, resolved into two peaks; one peak was always present and sedimented at about 4.6 S, the other sedimented at 5.5 S and was detectable in grown and secretive oviducts (Fig. 4A). Plasma labeled 17β-estradiol binding molecules always sedimented at about 4.0 S (Fig. 4B).

Figure 5 shows the effects of 17β-estradiol injection on the distribution of labeled 17β-estradiol binding molecules six hours after hormone administration. Estrogen induced decrease of RU and RF in the cytosol, and of RU in nuclean extract ($P < 0.01$). Instead, RF in nucleen extract inereased slightly. 17β-estradiol effect was similar twenty-four hours after the injection (not shown).

Figure 6 reports the effects of ovariectomy and sex hormone chronic administration on labeled

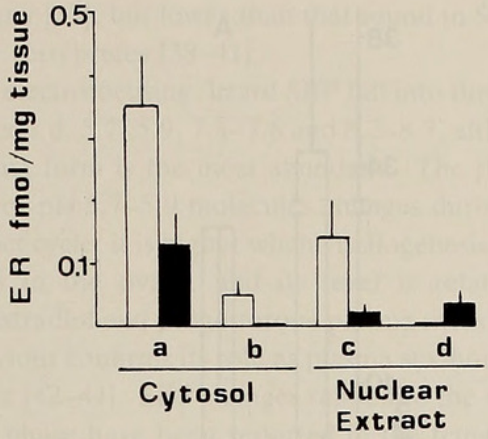

FIG. 5. Unfilled (□) and filled (■) binding sites (ER) concentration (fmol/mg tissue) in the cytosol and nuclear extract of *Podarcis s. sicula* secretive oviduct. a & c = animals injected with solvent; b & d = animals injected with 17β-estradiol. Bars indicate the S.E.

17β-estradiol binding molecules. Ovariectomy was followed by the RU decrease both in the oviduct cytosol and nuclear extract ($P < 0.01$), as well as by the RF increase in the cytosol ($P < 0.01$). In the oviduct of spayed females injected with 17β-estradiol or 17β-estradiol plus progesterone, a further RU and RF decrease occured in the cytosol ($P < 0.01$), whereas both RU and RF increased in the nuclear extract ($P < 0.01$). Progesterone administration induced a small RU decrease and a RF increase in the nuclear extract.

DISCUSSION

In the oviparous lizard, *Podarcis s. sicula*, seasonal oviduct growth and activity depend on ovarian steroids, namely 17β-estradiol and testosterone [7]. Proteins binding to these hormones have been found in the oviduct cytosol, and, during the breeding period, in the nuclei [8]. When injected *in vivo* into spayed females, however, 17β-estradiol is retained in the oviducal tissues, whereas testosterone is not; therefore the estrogen is supposed to play a leading role in physiological oviduct regulation [8]. Our data corroborate this assumption since we identified an 17β-estradiol receptor (ER) in the lizard oviduct which behaves according to the oviduct annual cycle.

17β-estradiol binding molecules bind the ligand with high affinity ($K_d = 7.0 \times 10^{-10}$ M for the cyto-

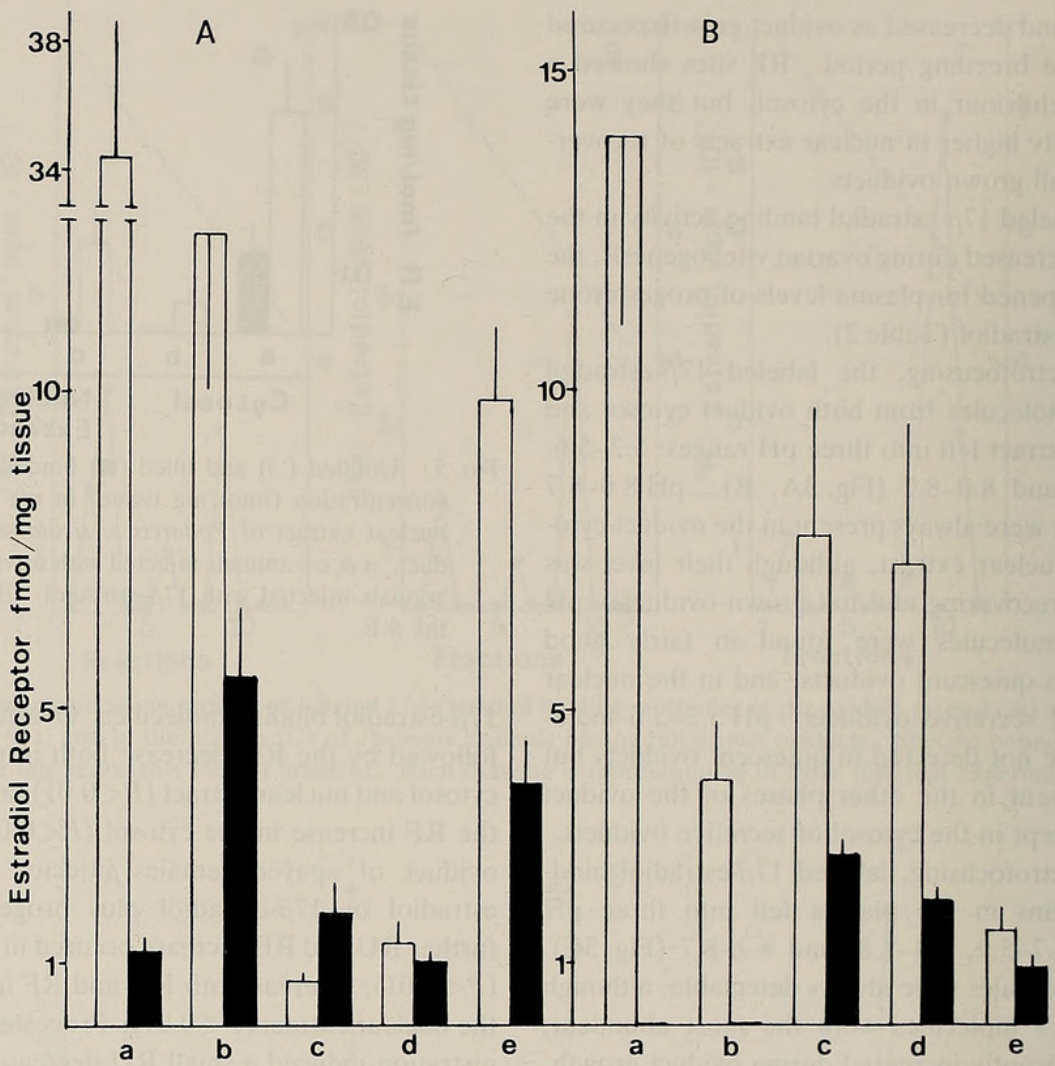

Fig. 6. Effects of ovariectomy and 17β-estradiol and/or progesterone treatment on unfilled (□) and filled (■) 17β-estradiol binding sites concentration in the oviduct cytosol (A) and nuclear extract (B) of *Podarcis s. sicula* captured in October. a=intact lizards; b=ovariectomized lizards treated with solvent (saline); c=ovariectomized lizards treated with 17β-estradiol; d=ovariectomized lizards treated with 17β-estradiol plus progesterone; e=ovariectomized lizards treated with progesterone. Bars indicate the S.E.

sol and 4.9×10^{-10} M for the nuclear extract). Ligand affinity does not significantly change during the cycle. These parameters are consistent with the properties of the oviduct 17β-estradiol receptor (ER) of several lower vertebrate species: elasmobranchs [14]; reptiles [15]; birds [16–19], and of the mammalian oviduct and uterus [20].

However, in the cytosol of full-grown oviduct, the specific 17β-estradiol binding resolves in an abnormal profile of the Scatchard plot (not shown), which does not allow K_d calculation. The meaning of this result is obscure.

The hormone-binding sites are present in the cytosol and nuclei of oviduct tissues. Binding activity is decreased by ovariectomy and is restored in spayed females after 17β-estradiol treatment. In spayed 17β-estradiol treated females, moreover, cytosolic unfilled and filled binding sites decrease whereas nuclear filled sites increase. In oviducts of female treated with 17β-estradiol for a short time, a displacement of the binding sites from the cytosol to the nucleus occurs, although the total amount appears to be decreased, as reported in several other studies [20, 21].

In our opinion this behaviour rules out an artifactual origin of cytosol binding sites as shown in several vertebrate systems [22, 23]. Quite a similar model has been reported for liver ER of the

salmon [24] and *Rana esculenta* [21].

The level of 17β-estradiol binding sites changes during the oviduct cycle. In the cytosol, it is higher in quiescent oviducts, but decreases as the organ grows. During growth a significant increase of nuclear filled sites occurs, which is coupled with an increase in plasma 17β-estradiol and progesterone titres as well as plasma levels of steroid binding proteins (SBPs).

In *Podarcis s. sicula* oviducts, the increase in 17β-estradiol-induced binding sites is not counteracted by progesterone administration, as reported for avian and mammalian systems [25–27]. This result, however, is in line with that obtained in the oviduct of the turtle *Trachemys scripta* [28], and proposes a difference in 17β-estradiol receptor regulation between higher vertebrates and lizards.

On sucrose gradient 17β-estradiol binding molecules resolve into two discrete peaks with sedimentation coefficients of 4.6 S and 5.5 S. The 4.6 S peak is always present, whereas the 5.5 S peak has been found only in the nuclear extract of growing or secretive oviducts, and, therefore, might represent an activated form of the 17β-estradiol receptor. Changes in the ER sedimentation coefficient following activation have been reported [29–31]. The sedimentation coefficient values of lizard oviduct putative ER are consistent with those found for ER from several vertebrate systems, sedimented at high ionic strength [32].

At electrofocusing, the oviduct 17β-estradiol binding molecules fall into three discrete pH ranges, id. 5.2–5.6, 7.0–7.7, and 8.0–8.7. It is difficult to interpret these results, although the absence of 5.2–5.6 molecules in quiescent oviducts and their appearance in the nuclear compartment of growing and secretive oviducts suggest that they might represent the activated receptor. A change in receptor pI following activation has been found in the androgen receptor of the rat prostate [33].

In *Podarcis s. sicula* plasma, 17β-estradiol binding proteins (SBPs) behave as those found in the plasma of other lower vertebrates [34–36], although in *Podarcis s. sicula,* binding is displaced also by corticosterone, a property found only in plasma SBP of *Nerodia sipedon* [37]. The sedimentation coefficient of lizard SBP is 4.0 S, a value similar to that reported for SBP of *Alligator mississipiensis* [35], but lower than that found in SBP of other vertebrates [38–41].

At electrofocusing, lizard SBP fall into three pH ranges, i.d. 5.7–5.9, 7.4–7.8 and 8.2–8.7, although the first form is the most abundant. The plasma level of pH 5.7–5.9 molecules changes during the oviduct cycle; it is higher when vitellogenesis progresses in the ovary, and its level is related to 17β-estradiol and progesterone plasma titres. This behaviour confirms its role as plasma sex-hormone carrier [42–44]. SBP changes related to the sexual cycle phase have been reported in the females of *Taricha granulosa* [45], *Alligator mississipiensis* [35] and in the male of *Podarcis s. sicula* [36].

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