Journal of Steroid Biochemistry, 1974, Vol. 5, pp. 687–691. Pergamon Press. Printed in Great Britain

INTERACTION OF 17β-ESTRADIOL AND TESTOSTERONE WITH THE OVIDUCT OF THE LIZARD *LACERTA SICULA*

VIRGILIO BOTTE, GIOVANNA GRANATA and CELESTE CRISTOFARO

II Chair of Comparative Anatomy, Institute of Histology, Faculty of Sciences, University of Naples, Italy

(*Received* 4 *February* 1974)

SUMMARY

In the lizard, *Lacerta sicula*, oviduct is stimulated in spayed females by the administration of 17β-estradiol or testosterone. The experiments *in vivo* show that the oviduct retains radioactivity only in the animals injected with [^{3}H]-17β-estradiol. *In vitro*, however, a specific binding of both hormones to oviduct-cytosol has been observed. It is not known if these sex hormones bind to the same macromolecule.

INTRODUCTION

It has been shown that 17β-estradiol or testosterone stimulate the oviduct of spayed females of the lizard, *Lacerta sicula*[1]. After the hormonal treatment, the chronological sequence of biochemical events, like the synthesis of DNA, RNA and proteins, is similar to that determined in mammalian target organs[2–4].

Since the conversion of testosterone to estrogens by the oviduct appears to be, at least *in vitro*, negligible (Botte, unpublished data), the question arose as to whether the two hormones, which show antagonistic effects in other reptilian organs[5], might, on the contrary, have a similar mechanism of action in the oviduct. In the present work the interaction of these hormones with the oviduct, *in vivo* and *in vitro*, has, therefore, been studied.

MATERIALS AND METHODS

Animals. Adult females of *Lacerta sicula* were obtained from the surroundings of Naples between September 1972 and March 1973 (in this period the gonads of the lizards are quiescent). The animals were ovariectomized as described elsewhere[1] and maintained thereafter in an animal room at 28 ± 4°, under a natural photoperiod. Water and food (*Tenebrio molitor* larvae) were supplied *ad lib.* The animals were used 15–20 days after castration.

Chemicals. [^{3}H]-17β-estradiol (S.A., 100 Ci/mmol) and [^{3}H]-testosterone (S.A., 85 Ci/mmol) were purchased from The Radiochemical Centre, Amersham, England. Non-radioactive steroids were obtained from Sigma (St. Louis, U.S.A.) and were checked for purity by thin layer chromatography on Silica gel. Soluene-100 and Instagel were purchased from Packard (Milano, Italy); Norit A charcoal from Sigma; Ribonuclease A, electrophoretically purified Deoxyribonuclease I, and Protease from Worthington (U.S.A.). Other chemicals were analytical grade and used without further purification.

Estimation of protein concentration. Protein concentration was measured by the method of Lowry *et al.*[6], using bovine serum albumin as a standard.

Scintillation counting. Radioactive samples were counted in a Packard Tri-Carb liquid scintillation spectrometer, Model 3220, with 10 ml Instagel. Counts per minute were converted to d.p.m. by the external standardization method.

Experimental

(a) *Uptake studies.* Two groups of 16 lizards each were injected subcutaneously with 1·9 ng of [^{3}H]-17β-estradiol or with 1·9 ng of [^{3}H]-testosterone, dissolved in 0·05 ml of saline. Groups of four animals were killed 2, 4, 8 and 12 h after the hormone administration; the oviducts, fragments of liver and of thigh muscles taken from each animal were weighed and digested with 0·5 ml of Soluene-100 at room temperature. Ten millilitres of Instagel were then added and vials were allowed to stand for 2 days at 4° before counting. The uptake of radioactivity was expressed as d.p.m./mg wet tissue.

(b) *Incubation of tissue extracts and charcoal adsorption.* The oviducts collected from 10–20 lizards were

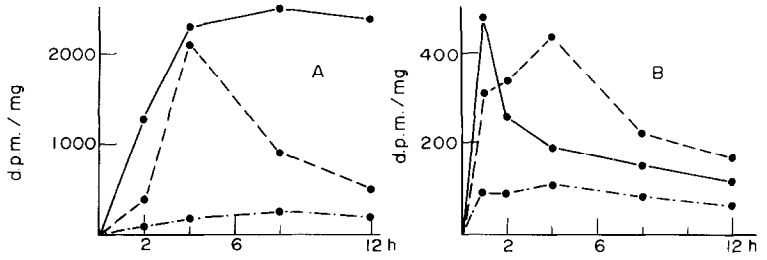

Fig. 1. Tissue tritium levels after a single subcutaneous injection of 1·9 ng of [^{3}H]-17β-estradiol (A) or [^{3}H]-testosterone (B) to adult spayed females of *Lacerta sicula*. Each point is based on 4 different determinations. (— —) oviduct; (— · — ·) liver; (· · ·) thigh muscles.

pooled and homogenized in a glass homogenizer with 100 mM Tris–HCl buffer, pH 7·4, containing 1·5 mM EDTA (TE buffer), in the ratio of 180 mg wet tissue per ml. The homogenate was centrifuged at 4° for 90 min at 180,000 *g* in a IEC B60 centrifuge and the sediment discarded.

The assay of binding was carried out by incubating 0·1 ml of the high speed supernatant with increasing amounts of labelled 17β-estradiol or testosterone (3·3 to 1020 pg) always dissolved in 0·1 ml of TE buffer. TE buffer was then added up to a final volume of 0·5 ml. Incubations were carried out for 2 h at 4°; 0·5 ml of a suspension of 0·1% Norit A charcoal in 0·1% Dextran dissolved in buffer were then added. Charcoal-free supernatants were obtained by spinning the mixture after 30 min at 1500 rev./min for 5 min. The supernatants were transferred into scintillation vials and radioactivity was counted as described above. Results were expressed as the ratio between bound radioactivity and radioactivity present in the incubation medium (bound d.p.m./medium d.p.m.) In many instances the values were plotted according to Scatchard[7].

In the experiments performed to test any competitive effect, 0·2 μg of non-radioactive steroids were added to the reaction mixture together with the tritiated hormones, and incubation was carried out as described above.

(c) *Enzymic digestion of the cytosol.* Aliquots (0·6 ml) of the cytosol were incubated for 2 h at 25° with 0·5 ml of TE buffer containing 144 μg of Ribonuclease A, Deoxyribonuclease or Protease respectively. The incubation mixtures were then cooled and the extent of binding was assayed on aliquots of 0·2–0·4 ml by the method described above.

(d) *Gel filtration.* Gel filtration was performed at 4°C, with Sephadex G-200 columns (1 × 27 cm.) equilibrated with sodium phosphate buffer, pH 7·4. The same buffer was used for elution and 1 ml fractions were collected at a flow of 4 ml/h. Proteins were

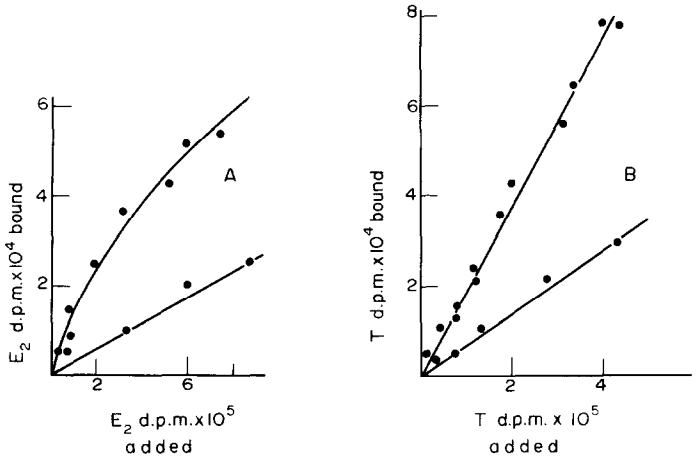

Fig. 2. Binding of [^{3}H]-17β-estradiol (A) or [^{3}H]-testosterone (B) to lizard oviduct cytosol. Lower curves indicate the competition on this binding affected by 17β-estradiol (A) or testosterone (B). Incubations were carried out at 4°C for 2 h; the charcoal technique was used to adsorb unbound steroids.

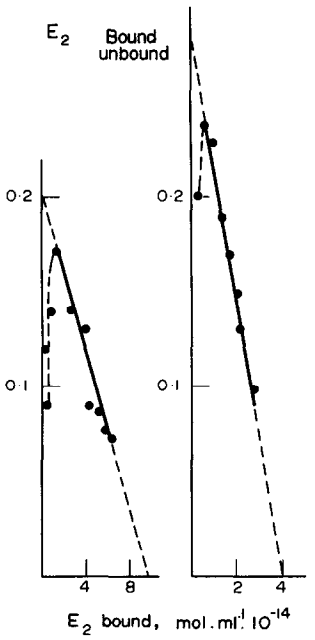

Fig. 3. Scatchard plots of two different experiments of binding of [^{3}H]-17β-estradiol to cytosol of lizard oviduct.

recorded at 280 nm; each fraction was then poured in a counting vial containing 10 ml Instagel.

RESULTS

The tritium levels detected in oviduct, liver and thigh muscles after a single injection to the animals of [^{3}H]-17β-estradiol (A) or [^{3}H]-testosterone (B) are reported in Fig. 1. As one can see, the oviduct takes up and retains the radioactivity only when 17β-estradiol is administered; unless testosterone is quite rapidly released, it does not bind to the oviduct.

In Fig. 2 the results of the binding of labelled 17β-estradiol (A) and testosterone (B) to the oviduct cytosol are shown. Both hormones bind to the high speed cytosol. The extent of binding was decreased by non-radioactive estradiol (lower curve in A) and non-radioactive testosterone (lower curve in B). The Scatchard plots of the values obtained from 6 determinations on oviducts collected from animals captured in different periods of time gave a K_{ass} ranging around 10^{10}. Two Scatchard plots of the experiments with [^{3}H]-17β-estradiol are shown in Fig. 3. Similar results have been obtained with [^{3}H]-testosterone. Scatchard plots of two separate experiments are presented in Fig. 4.

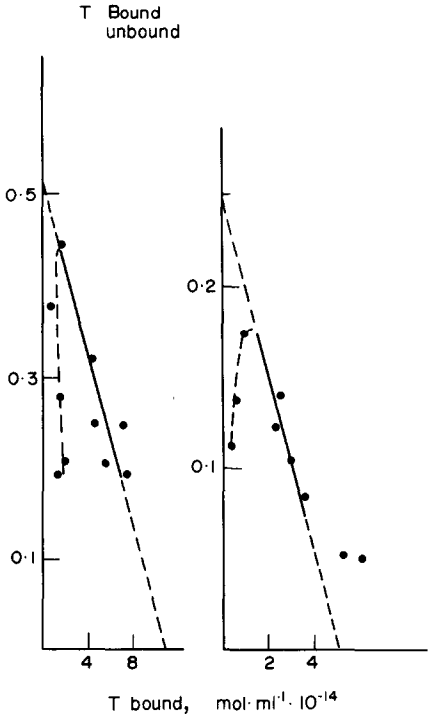

Fig. 4. Scatchard plots of two different experiments of binding of [^{3}H]-testosterone to cytosol of lizard oviduct.

Table 1. Competition of some steroids for [^{3}H]-17β-estradiol and [^{3}H]-testosterone binding to cytosol of lizard oviduct

Non-radioactive steroids (0·2 μg)	[^{3}H]-17β-estradiol bound (d.p.m./μg protein)*	[^{3}H]-testosterone bound (d.p.m./μg protein)*
0	1400	1200
17β-Estradiol	180	1215
17α-Estradiol	1200	1215
Estrone	1400	1160
Estriol	1080	1450
Testosterone	1310	100
17β-Hydroxy-5α-androstan-3-one		180
Cortisol		1180

* Mean values of three different experiments. The values of each experiment were within 15% of the mean value.

Table 2. Effects of several enzymic activities on the binding of [^{3}H]-17β-estradiol and [^{3}H]-testosterone to the cytosol of lizard oviduct

Enzymes	Non-radioactive steroids	(A) [^{3}H]-17β-estradiol bound (d.p.m./μg prot.)*	(B) [^{3}H]-testosterone bound (d.p.m./μg prot.)*
0	0	344	549
0	0·2 μg 17β-estrad.	60	
RNAase A	0	324	546
DNAase	0	320	500
Protease	0	80	300
0	0·2 μg testosterone		250

* The figures are mean values of two different experiments.

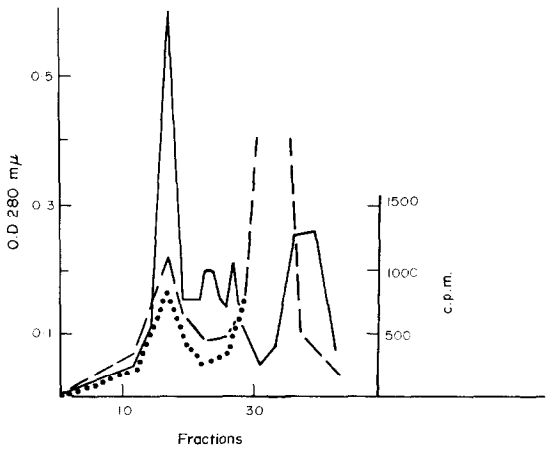

Fig. 5. Sephadex G-200 filtration of [^{3}H]-17β-estradiol (———) and [^{3}H]-testosterone (· · · ·)-cytosol complexes. The figure is based on the results obtained with two equivalent columns; [^{3}H]-17β-estradiol–cytosol complex was applied to the first one; [^{3}H] testosterone–cytosol complex to the second. In both cases the elution pattern of proteins (– – –) was very similar; therefore, it has been reported only once, using a single symbol.

The binding of labelled 17β-estradiol to the oviduct cytosol seems to be a very specific process, since only non-radioactive 17β-estradiol competes for binding sites; a limited competition occurred also in the case of estriol. The binding of labelled testosterone to cytosol was inhibited only by testosterone and 17β-hydroxy-5α-androstan-3-one (androstanolone). Other steroids tested did not show any competitive effect (Table 1).

The 17β-estradiol- or testosterone-binding to cytosol was sensitive to the action of proteolytic enzymes but not of nucleases; this would indicate that an important part of the molecule is proteic in nature (Table 2).

Gel filtration of hormone-cytosol complexes through Sephadex G-200 showed that the complexes are eluted with the void volume of the columns (Fig. 5). By using equivalent columns, very similar elution patterns were obtained when [^{3}H]-17β-estradiol or [^{3}H]-testosterone had been incubated with cytosol.

DISCUSSION

The presence in the cytosol of the lizard, *Lacerta sicula*, oviduct of proteic complexes able to bind 17β-estradiol and testosterone is supported by the experiments presented here. The specificity of binding is higher than that observed in the case of mammalian target organs. The [^{3}H]-17β-estradiol-cytosol binding appears to be affected in lizard oviduct only by 17β-estradiol and, to a lesser extent, by estriol, whereas in mammalian target organs several estrogens and antiestrogens compete with 17β-estradiol for binding sites[8–10]. The [^{3}H]-testosterone–cytosol binding in lizard oviduct is inhibited by testosterone and 17β-hydroxy-5α-androst-3-one; in the case of mammalian prostate cytosol, not only these steroids but also some estrogens compete with 17β-estradiol for binding sites[11].

The K_{ass} of 17β-estradiol or testosterone binding to cytosol of the lizard oviduct ranged in the order of 10^{10}. This value is similar to those determined for steroid receptors in mammals[12, 13]. It has to be pointed out, however, that the yield obtainable by the charcoal technique is around 50–60% that of equilibrium methods[14]. No comparative data are available to test the reliability of the charcoal method in the calculation of the association constants. For these reasons the value reported is to be considered only indicative.

The absence of cross reaction between 17β-estradiol and testosterone binding to the cytosol might indicate that discrete protein complexes do exist in lizard oviduct, capable of binding the two hormones. On the other hand, gel filtration through Sephadex G-200 seems to exclude this possibility. These results call, however, for more studies using other separation methods.

The physiological significance of the presence of the binding capacity for 17β-estradiol and testosterone in the lizard oviduct is not known. It is noteworthy that testosterone is not retained by the oviduct *in vivo*, and that it has not been identified in lizard ovarian extracts[15]. No data, moreover, are available on the steroids present in the blood of the lizard during the circannual cycle.

Acknowledgements—This work was in part supported by a grant to II Chair of Comparative Anatomy of Consiglio Nazionale delle Ricerche, Italy.

REFERENCES

1. Botte V.: *Monitore Zoologico Italiano* **8** (1974) 47–54.
2. Botte V.: *Boll. Zool.* **40** (1973) 305–314.
3. Jensen E. V. and DeSombre E. R.: *Current Topics in Experimental Endocrinology* (Edited by Martini L. and James V. H. T.). Academic Press, New York, Vol. 1 (1971), pp. 229–269.
4. Mueller G. C., Vonderhaar B., Kim U. H. and Le Mahieu M.: *Recent Progr. Hormone Res.* **28** (1972) 1–49.
5. Botte V. and Delrio G.: *Gen. comp. Endocr.* **9** (1967) 110–115.
6. Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J.: *J. biol. Chem.* **193** (1951) 265–275.
7. Scatchard G.: *Ann. N.Y. Sci.* **51** (1949) 660–672.
8. Korenman S. G.: *Steroids* **13** (1969) 163–177.
9. Korenman S. G.: *Endocrinology* **87** (1970) 1119–1123.
10. DeSombre E. R., Chabaud J. P., Puca G. A. and Jensen E. V.: *J. steroid Biochem.* **2** (1971) 95–103.
11. Fang S., Anderson K. M. and Liao S.: *J. biol. Chem.* **244** (1969) 6584–6595.
12. Puca G. A., Nola E., Sica V. and Bresciani F.: *Biochemistry* **10** (1971) 3769–3780.
13. Thomas P. J.: *J. Endocr.* **57** (1973) 333–359.
14. Jungblut P. W., Hughes S., Hughes A. and Wagner R. K.: *Acta endocr., Copenh.* **70** (1972) 185–195.
15. Lupo di Prisco C., Delrio G. and Chieffi G.: *Gen. comp. Endocr.* **10** (1968) 292–294.