Atti Accademia Nazionale dei Lincei

Classe Scienze Fisiche Matematiche Naturali

Rendiconti

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Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Serie 8, Vol. **62** (1977), n.3, p. 387–391.

Accademia Nazionale dei Lincei

<http://www.bdim.eu/item?id=RLINA_1977_8_62_3_387_0>

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Accademia Nazionale dei Lincei, 1977.

SEZIONE III

(Botanica, zoologia, fisiologia e patologia)

Biologia. — *Receptors for sex hormones in the skin and oviduct of* Triturus cristatus carnifex (*). Nota di MICHELA D'ISTRIA, GIOVANNI DELRIO e FRANCA CITARELLA, presentata (**) dal Socio G. MONTALENTI.

RIASSUNTO. — Nell'ovidutto e nella pelle di femmine di *Triturus cristatus carnifex* sono stati trovati recettori citoplasmatici per gli ormoni steroidei sessuali. Nell'ovidutto è stata osservata la presenza di un recettore per l'estradiolo-17 β solo negli animali catturati nei mesi di Aprile-Luglio, mentre nella pelle è stato messo in evidenza un recettore per il testosterone in diversi periodi dell'anno.

INTRODUCTION

The administration of testosterone propionate to intact and castrated females of *Triturus cristatus carnifex* induces the appearance of male sex characteristics on the skin (dark spots, etc.) and stimulates the oviducts (Galgano, 1942 a). Already in 1933 and 1942 De Beaumont and Galgano respectively proposed that the ovary was in some way involved in the control of growth of the oviduct but the precise role of ovarian secretion in this regard is still very poorly understood. According to Galgano the mammalian follicular or luteinic hormones proved to be ineffective in inducing *Triturus* oviduct growth; these hormones, however, stimulated the height of the tail.

Recently we succeded in finding sex hormone receptors in the skin of male *Triturus cristatus* and the skin and thumb pads of *Rana esculenta* (d'Istria *et al.*, 1975, Delrio and d'Istria, 1973).

Thus, in this work an effort has been made to study the retention of labelled testosterone and 17 β-estradiol by the oviduct of *Triturus cristatus carnifex*, and to show the presence of any sex hormone receptors in the female skin and oviducts.

MATERIALS AND METHODS

Adult females of *Triturus cristatus carnifex* caught in the vicinity of Naples in different periods of the year were castrated and used for this study two weeks after the operation. H^3-testosterone (86 Ci/mM) and H^3-17 β-estradiol (100 Ci/mM) from the Radiochemical Center (Amersham, England) were

(*) Research performed under CNR project " Biology of Reproduction " at the II Chair of Zoology, University of Naples, Via Mezzocannone 8, 80134 Naples (Italy).

(**) Nella seduta del 12 marzo 1977.

used. In these uptake studies the technique of Fang *et al* (1969) was followed using groups of 25 animals injected with 0.5 μCi of labelled steroids in saline solution. For the receptor studies oviducts and skin taken from different zones, ventral, dorsal, lower jaw and limbs of castrated animals, were minced and homogenized in 1.5×10^{-3} M EDTA, 3×10^{-3} M Dithiothreitol, 2×10^{-2} M Tris-HCl buffer pH 7.5 in an all-glass homogenizer. The homogenate was centrifuged at $600 \times g$ for 10 min and the supernatant was centrifuged at $105.000 \times g$ for 60 min. in an IEC mod. B 60 centrifuge (see Delrio and d'Istria 1973, for details). The influence of temperature on steroid binding was tested at 0°, 10°, 20°, 37° and 60 °C at pH 7.5; the effect of pH was studied over the range 2.5–10. The gut was used as control tissue. The data of all experiments were adjusted to correspond to a protein concentration of 1 mg/ml. The enzymic digestion on 100 μl (200 μg of protein) of $105.00 \times g$ supernatant with radioactive estradiol, previously bound *in vitro*, was carried out with pronase (Sigma), DNAase II and RNAase (Worthington) for 1 h at 37 °C. The separation of free and bound steroids was performed by competitive adsorption by Dextran-coated charcoal (Delrio and d'Istria, 1973).

Results and Discussion

The retention curves with H^3-17 β-estradiol were obtained using the supernatant at $105.000 \times g$ from the oviduct homogenate. As shown in Table I cold 17 β-estradiol and estrone cause a reduction of about 80 % in binding while cold testosterone causes a diminution of about 50 %. Using the Scatchard plot we obtained a K_{ass} ranging between 1.19 and $2.45 \times 10^9 M^{-1}$ and a number of sites of 1.6 and 4.5×10^{-10} M (fig. 1). No specific retention was observed with H^3-testosterone. These results were obtained with animals caught in April-June, i.e. during the breeding season which, according to Galgano (1942 b), corresponds to maximal ovarian secretion. In a similar experiment carried out on the animals of November-December, when the ovaries are filled with almost ripe eggs and ovarian hormonal secretion is just beginning, no estrogen binding macromolecule supernatant was evidenced in $105.000 \times g$.

On the contrary in the skin from different zones of these animals an androgen receptor was detected in the $105.000 \times g$ supernatant: a marked competition was observed with testosterone and dihydrotestosterone, while binding was not affected by cyproterone acetate (CPA, an antiandrogen), estrogens, progesterone and corticoids. The K_{ass} is about $1.7 \times 10^9 M^{-1}$ and the number of sites is about 8×10^{-10} M. In both the tissues studied the binding of 17 β-estradiol and testosterone have an optimum temperature between 10° and 20 °C at pH 7.5. In the enzymic digestion experiments with Pronase, DNAase and RNAase only the pronase causes a loss of binding of about 50 %. No retention curves were obtained with labelled testosterone and estradiol in the gut supernatant.

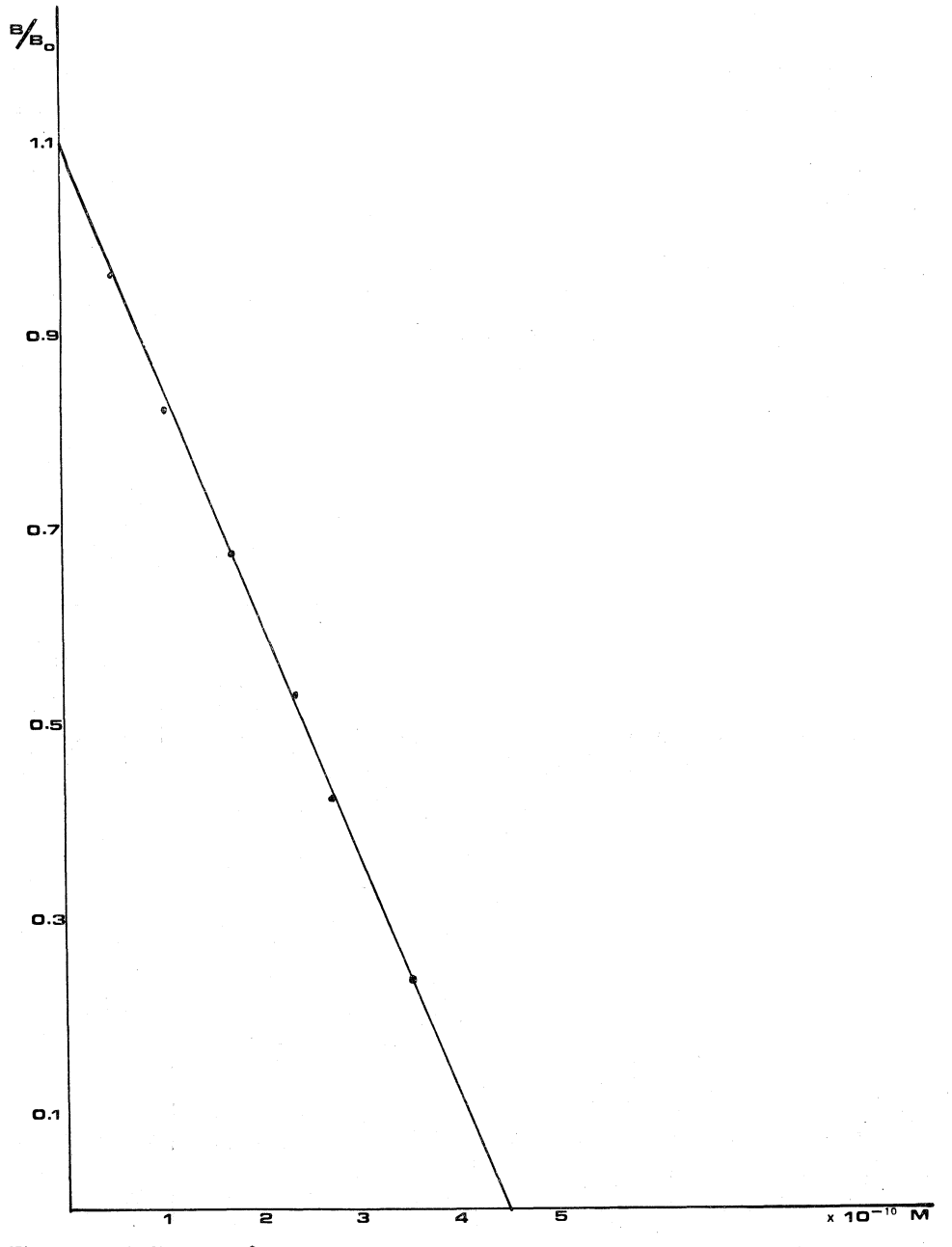

Fig. 1. – Binding of H^3-estradiol at various concentrations by oviduct 105.000×g cytosol during overnight incubation in 1.5×10^{-3} M EDTA, 2×10^{-2} M Tris-HCl buffer pH 7.5 at 4 °C. The values are adjusted to a protein concentration of 1 mg/ml and plotted after Scatchard analysis.

TABLE I

Reduction (%) *in cytosol binding of labelled steroids in* Triturus cristatus carnifex.

Competing hormones	Concentration (*n*M)	Tissues	
		Oviduct (E_2—H^3)	Skin (T—H^3)
Estradiol-17 β	1.3	80	0
Estrone	1.3	80	0
Testosterone	1.4	50	85
Dihydrotestosterone	1.4	—	85
Progesterone	1.1	0	0
Cortisol	1.1	0	0
Cyproterone acetate	2.0	—	0

— not tested; E_2 = 17 β-estradiol; T = Testosterone.

Steroid hormone receptors were found in the oviducts of other species of non-mammalian vertebrates: O'Malley *et al.* (1970) evidenced a progesterone receptor in chick oviduct; Botte *et al.* (1974) observed a specific binding of testosterone and 17 β-estradiol to the oviduct cytosol of *Lacerta sicula*. In both cases, however, the oviducts were responsive to testosterone and estradiol in *Lacerta* and to progesterone in chick. Galgano (1942 *a*) failed to observe a stimulation of the oviduct in *Triturus* after administration of estradiol to the animals caught during a phase of low ovarian secretion, at the time when an estrogen receptor is absent in the oviduct. At the same time the stimulatory effects of testosterone on the unresponsive estradiol oviducts are rather difficult to account for since no testosterone receptor was ever observed. However, it can be pointed out that in April the oviducts are able to take up both H^3-17 β-estradiol and H^3-testosterone, although the latter in a smaller amount. In *Pleurodeles waltlii* Martin and Ozon (1969) observed that the Müllerian ducts concentrate the H^3-17 β-estradiol and *in vitro* uptake is lowered in the order of 58 % by 17 β-estradiol and 25 % by testosterone. These authors, however, did not mention the reproductive status of their animals.

The occurrence of male sex characteristics in the skin of intact and castrated females of *Triturus cristatus*, following the administration of testosterone proprionate (Galgano, 1942 *a*) indicates the presence of an androgen receptor in the female skin.

It must be pointed out, however, that in the skin of the male newt only an estradiol-receptor was demonstrated; however, estradiol stimulates only tail height while the other characteristics of the skin are testosterone dependent.

Binding macromolecules for heterosexual sex hormones in both females and males have also been demonstrated in mammals. Thus the cytosol of uterine tissue from immature rats (Giannopoulos, 1971) showed the presence of a testosterone binding protein and McCann *et al.* (1970) found an estradiol cytoplasmic receptor in the calf prostate and seminal vescicle. It seems that heterosexual hormones are ineffective and that this can be explained by the absence of a nuclear receptor responsible for hormonal transport on DNA. The autoradiographic studies of Stumpf (1970) seem to further support this hypotheisis. However, in mammals the presence of a receptor for heterosexual hormones is always associated with the presence of receptors for homeosexual hormones.

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