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Sex steroids and postreproductive refractoriness in the lizard, *Podarcis s.sicula*

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ABSTRACT

The evolution of sex steroids in the plasma and gonads of the lizard *Podarcis s. sicula* during the postreproductive period shows that these hormones could be involved in determining refractoriness. In the male, during this phase, the oestradiol could lower the hypothalamo-hypophyseal system through a negative feed-back. In the female a similar role could be assigned to progesterone.

KEY WORDS: Lizard reproduction; refractoriness; sex steroids.

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INTRODUCTION

In the lizard, *Podarcis s. sicula* from peninsular Italy the annual breeding activity is restricted to spring. The gonads and the secondary sexual characters (SSC) develop at the beginning of the spring and are functional until the end of July when a fast regressive phase begins (Licht *et al.*, 1969; Angelini *et al.*, 1976, 1980, 1981). In summer however, this stimulatory effect of ambiental cues declines owing to the setting in of refractoriness which is followed by the complete regression of gonads and SSC. This refractory status assumes a considerable ecological value. In fact, the precocious interruption of reproductive activity, when some ambiental cues like temperature seem to be still favourable, prevents the presence of new-hatched lizards in early autumn in a perspectively unfavourable period (Angelini *et al.*, 1981; Angelini & Ghiara, 1984).

The morphological modifications of gonads and genital tracts due to refractoriness are quite peculiar. In the male, testicular spermatogonial mitoses rapidly decrease and almost all spermatocytes I and II, spermatids and sperms degenerate. In the reduced seminiferous tubules only spermatogonia and Sertoli cells persist (Angelini *et al.*, 1980). Leydig cells progressively shrink to become indistinguishable from interstitial fibroblasts (Varano *et al.*, 1973). The epithelial cells of the epididymis appear flattened and devoid of secretory granules; in the lumen both secretion and sperms are lacking.

In the female, the ovary does not contain any vitellogenetic oocytes; the oviduct is significantly reduced in size and its glands are small and without secretion (Botte, 1973).

Refractoriness lasts until late autumn when all the components of genital apparatus and SSC develop again if the animals are maintained for a few weeks in terraria with high temperature and long photoperiod. Refractoriness, moreover, seems to be largely endogenously regulated. Any manipulation of photothermal regimes, in fact, can only anticipate or postpone but never prevent its appearance (Angelini *et al.*, 1976).

The endogenous mechanisms initiating and maintaining refractoriness are still scarcely known. Indirect proofs sustain an involvement of pituitary gonadotropins which regulate the seasonal development and activity of the gonads (Della Corte *et al.*, 1968). More recently, some of us have shown that in both sexes the refractory gonads contain some unknown lipidic substances which, whenever injected to non-refractory lizards in early spring, hamper gonad recrudescence and SSC development (Angelini *et al.*, 1981).

These observations have led us to the supposition that the gonad itself could induce the refractory status by producing some kinds of steroid hormones after the breeding period. These, in turn, could act on the hypothalamo-hypophyseal axis by inhibiting it through a negative feed-back.

To test this hypothesis sex hormones have been eva-

luated in the plasma during the postreproductive period and in refractory gonads. The action of continuous administration of some sex steroid on the gonads and genital tracts of reproductive lizards has also been tested.

MATERIALS AND METHODS

Animals. We utilized male and female of *Podarcis s. sicula* captured during reproductive and postreproductive periods in the neighbourhood of Naples: the average weight was g 11.39 ± 0.87 for males and 6.58 ± 0.60 for females.

Blood was collected from the heart by means of heparinized tubes; the plasma obtained after centrifugation was preserved at − 80°C until use. Samples of gonads and genital tracts (epididymis in the males and oviduct in the females) were fixed in Stieve's fluid to be utilized later for histology.

During all of the experimental treatments, the lizards were maintained in open terraria with ambient photothermal regimes (in May L:D 14-16: 8-10 hrs; temperature 16°-18°C). Food (meal worms and fresh vegetables) was given *ad libitum.*

Sex hormone assays Progesterone, testosterone and oestradiol were assayed in plasma samples by means of a RIA method (D'Istria *et al.,* 1974; Polzonetti *et al.,* 1984) whose reliability for lizard blood was previously ascertained. The following sensibilities were registered: progesterone, 7 pg (intrassay variability, 8%; interassay, 10%); oestradiol. 5 pg (intrassay, 7%; interassay, 13%); testosterone, 7 pg (intrassay, 9%; interassay, 13%). Since the antibody used for testosterone detection also interacted with dihydrotestosterone, the related data are reported as androgens.

Sex hormone determination in gonads. Three refractory gonad pools were utilized: *1.* testes of 42 males captured on 29th of July: *2:* testes of 55 males obtained on 10th of August; *3:* Ovaries of 71 females captured on 10th August. Tissues were extracted three times with cold 85% ethanol. The combined solvents were dried under vacuum and then chromatographed on silica gel plates in the system: petroleum ether-ethyl ether (96:4, v/v). The fraction containing steroids was then applied to a plate which was run in the system: esane-petroleum ether − acetic acid (90:10:1, v/v). The steroidal fraction was finally fractionated on plates developed in the system benzene-ethanol (90:10, v/v). Areas with R_f of progesterone, testosterone and oestradiol were eluted and the extracts were used for hormone content determination by the previously reported RIA method.

Experimental treatments. At the end of April 1982, 60 adult males and 60 females were divided in three groups. The animals of the first group were injected subcutaneously every week (May 1st, 7th, 14th and 21st) with 100 μg of testosterone propionate (Testoviron Depot, Schering) dissolved in 50 μl of almond oil. To the second group of lizards was administered the same amount of oestradiol valerate (Progynon Depot, Schering). The third one received only the oil. Four males and four females from each group were taken at random, weighed and sacrificed two days after each hormonal injection. Gonads and genital tracts (epididymis in the males and oviduct in the females) were then used for histological studies as previously reported.

In the same period of 1984, two experiments were carried out. In the first, 21 adult males were divided into 3 groups and treated as follows: the first group with oestradiol valerate, as in the previous experiment; the second with oestradiol valerate and mammalian FSH, 50 U.I. (Metrodin, Serono) dissolved in 100 μl of saline and injected intraperitoneally; the third group with oil and/or saline only. Two days after the last injection the animals were weighed and sacrificed. The gonads and genital tracts were excised and utilized for histological examinations as previously reported.

In the second experiment, 12 adult females were separated into two groups. The lizards of first group were injected twice, every week, with 50 μg of progesterone caprate (Proluton, Depot, Schering) dissolved in 100 μl of almond oil. As already described, the animals of the second group received the solvent alone. Three days after the last injection the lizards were weighed, sacrificed and utilized as reported above.

In all the experimental treatments, mortality was very low and never higher than that observed in the controls.

RESULTS

Table I reports the plasma levels of sex hormones through the postreproductive period. In the male, while the progesterone titre does not significantly change, androgens progressively decrease ($p < 0.01$). On the contrary the, oestradiol level, relatively low during May and June, appears high in July ($p < 0.01$). In the female, progesterone is higher in June ($p < 0.01$); androgen titres, always lower than in the male, do not significantly change during the postreproductive period; oestradiol progressively decreases from May to July ($p < 0.01$).

Table 2 reports the results of sex steroid determination in refractory gonads. It shows that these gonads still contain a certain amount of hormones. If the values reported in pg of hormone/g of fresh tissue are considered, the results concerning progesterone and oestradiol appear quite uniform for both sexes, whereas a higher amount of androgen is observed in ovarian extracts. The experimetanl treatments gave the following results. Hormonal administration did not cause a significant alteration in body weight (males: controls, 11.3 ±

TABLE I - *Plasma levels of sex steroids in the lizard,* Podarcis s. sicula, *during different phases of the reproductive cycle.*

Periods	Hormone titres (ng/ml of plasma) Progesterone	Androgens	Oestradiol
A. Males			
Breeding period (May)	(7) 5.31 ± 1.23	(6) 80.00 ± 21.30	(6) 0.235 ± 0.080
Breeding period (June)	(7) 5.44 ± 0.80	(6) 10.00 ± 2.50	(6) 0.372 ± 0.128
Refractory period (July)	(7) 5.14 ± 0.53	(6) 5.51 ± 1.54	(6) 1.603 ± 0.400
B. Females			
Breeding period (May)	(4) 77.41 ± 7.28	(5) 5.87 ± 3.00	(5) 1.639 ± 0.216
Breeding period (June)	(4) 67.25 ± 11.00	(5) 5.23 ± 3.14	(5) 1.055 ± 0.091
Refractory period (July)	(4) 3.53 ± 0.48	(5) 2.78 ± 0.69	(5) 0.602 ± 0.079

Mean values ± SE. The numbers in parentheses indicate the animals used for each dosage.

TABLE II - *Sex steroids in extracts of refractory gonads of the lizards,* Podarcis s. sicula.

Date		Tissue	N. of gonads	Pool weight in (g)	Sex hormones (ng/g fresh tissue) Progesterone	Androgens	Oestradiol
July,	29	Testes	84	1.20	13.34	24.29	9.70
August,	10	Testes	110	0.90	13.60	17.06	7.00
August,	10	Ovaries	142	1.20	12.50	37.32	8.50

0.9 g; treated with testosterone propionate, 9.48 ± 0.8; oestradiol valerate, 9.71 ± 0.79; females: controls, 6.58 ± 0.60; testosterone proprionate, 7.55 ± 0.34; oestradiol valerate, 7.88 ± 1.98; progesterone caprate, 8.00 ± 2.01).

In control males throughout the experiment, spermatogenesis was fully active, the Leydig cells large and filled with minute lipidic droplets and the epididymis well developed and secretory.

In oestradiol-treated males, after two hormonal injections, spermatogonial mitoses stopped and a reduction in spermio-histogenesis and spermiation rate was also observed. Conversely, the epididymis was still well developed and contained few sperms and several degenerated germinal cells (mainly spermatocytes II and spermatids) in the lumen. After the third oestradiol administration, spermatogenesis completely regressed; in most seminiferous tubules only Sertoli cells and spermatogonia were found. Leydig cells could not be distinguished from fibroblasts. The epididymis was completely reduced and affected by a connective sclerosis. Such an involute picture persisted for the rest of the experiment.

In oestrogen-treated males which also received mammalian FSH, the testes and epididymis were instead fully active for the whole experimental period.

In testosterone-treated males, sperm production was enhanced in the first two weeks. After three weeks, however, spermiogenesis progressively slowed down and finally was suppressed. In lizards treated for 4 weeks, only a few seminiferous tubules contained some spermatocytes and a few spermatids, as well as spermatogonia and Sertoli cells. Leydig cells, quite normal until the third experimental week, were indistinguishable from fibroblasts in the last observation. On the contrary, the epididymis remained hypertrophic.

In control females, two ovulatory waves took place, in the second and fourth week of May respectively. Ovarian morphology was consistent with these events. In fact, besides several medium-sized vitellogenetic oocytes, a few postovulatory follicles were found after the first week. Large oocytes were present in the second observation samples and again small vitellogenetic follicles and postovulation follicles in the third. Large oocytes were not observed in the ovaries of the last observation. Several oogonial mitoses were registered in the ovarian germinal layer. The oviduct appeared well developed, but at the end of the experiment showed some typical regressive phenomena in the tubal tract.

In progesterone-treated lizards, egg deposition was not observed. At autopsy, the ovary was devoid of vitellogenetic oocytes. The oviducts, well developed, contained in all 6 specimens, 4-6 eggs.

In testosterone-treated females the second deposition was also absent. Ovarian and oviducal morphology was very similar to that reported for oestradiol-treated animals. Oogonial mitoses, however, were suppressed from the third week and no yolk was deposited in primary oocytes.

In oestradiol treated females the second deposition was lacking, in fact, in the ovaries of lizards treated for two weeks, large vitellogenetic follicles were in athresia. Successively, vitellogenesis was suppressed, but some yolk was stored in several primary oocytes of the germinal layer. Oogonial mitoses were lacking throughout the experimental period. The oviduct, instead, always remained hypertrophic.

DISCUSSION

The results of our investigations allow some evaluation of the mechanisms involved in the determination of postreproductive refractoriness in the lizard, *Podarcis s. sicula.*

In the male, the circulating sex steroids throughout the postreproductive period show a progressive decrease of androgens, which is consistent with SSC regression. Moreover, in refractory lizards the increase of oestradiol is peculiar. Since sex hormones were found in refractory gonads, we suspect a direct implication of these organs in the determination of refractoriness.

In our working hypothesis, postreproductive testes produce increasing amounts of oestradiol which acts through a negative feedback mechanism of the hypothalamo-hypophyseal system by lowering the secretion of gonadotropin and eventually gonadal testosterone biosynthesis. Several proofs seem to substantiate this interpretation as already shown by other Authors (Botte & Del Rio, 1967). The administration of oestradiol to reproductive lizards induces some regressive phenomena in the testes and genital tracts which are similar to modifications observed during the refractory period. These effects are completely prevented by administration of mammalian FSH. Moreover, after the breeding period, marked lytic phenomena, involving the pituitary gonadotrophic cells (Della Corte *et al.,* 1968) and

the naturally occurring regressive processes can be postponed by the administration of a precocious mammalian FSH (Della Corte *et al.*, 1966).

In females, the role reported above could be tentatively assigned to progesterone which is still high in the plasma of lizards in June. In our opinion, this aspect cannot be related only to ovulations which are few during June in our control females (Angelini *et al.*, 1981; Angelini & Ghiara, 1984). This interpretation could be supported by our results which indicate an inhibitory action of progesterone on egg deposition and ovarian vitellogenesis when administered continuously to reproductive females. The experimental treatments with sex hormones, however, should be confirmed using more physiological doses to better ascertain their values.

Refractoriness could also be the expression of a reduced sensitivity of gonadal targets to gonadotropins. In fact, once the refractory period starts, exogenous gonadotropins show a limited action in restoring both spermatogenesis and SSC development (Botte & Angelini, 1980). In the female of the Iguanid, *Anolis carolinensis*, the presence of athretic follicles in postreproductive ovaries lowers the organ sensitivity to gonadotropins. This effect has been ascribed to an unknown steroid secreted by the athretic follicles (Crews, 1979).

In conclusion our data, though preliminary, support the hypothesis that refractoriness depends upon specific modification of the lizard endocrine system. These imply the activity of both hypothalamo-hypophyseal axis and of gonadal endocrine components.

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