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Are the gonads implied in postreproductive refractoriness determinism in the lizard, Podarcis s. sicula?

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Biologia. — *Are the gonads implied in postreproductive refractoriness determinism in the lizard,* Podarcis s. sicula ? (*). Nota di FRANCESCO ANGELINI, GAETANO CIARCIA, ORFEO PICARIELLO e VIRGILIO BOTTE, presentata (**) dal Corrisp. G. CHIEFFI.

RIASSUNTO. — Da gonadi di lucertole *Podarcis s. sicula* di entrambi i sessi, catturate durante il periodo di refrattarietà postriproduttiva, sono stati preparati estratti acquosi e lipidici. Questi iniettati in lucertole invernali non refrattarie, hanno impedito il regolare sviluppo delle gonadi indotto dalle alte temperature.

Nei maschi l'estratto acquoso ha determinato un arresto delle mitosi spermatogoniali e quindi la chiusura del ciclo spermatogenetico, mentre gli estratti lipidici hanno completamente inibito l'attività endocrina dal momento che hanno bloccato lo sviluppo dei CSS e le ultime fasi della spermatogenesi.

Nelle femmine solo gli estratti lipidici dell'ovario sembrano attivi nell'inibire sia la crescita dei follicoli che lo sviluppo dell'ovidutto.

Questi dati indicano che le gonadi di *Podarcis s. sicula* possono essere implicate nel determinismo della refrattarietà postriproduttiva.

The postreproductive refractoriness of genital apparatus to high temperature stimulation has a relevant ecological value in reptiles living in temperate zones (Licht *et al.*, 1969; Callard *et al.*, 1972; Fischer and Ewald 1972; Licht 1973; Angelini *et al.*, 1976, 1980; Botte and Angelini 1980). It in fact stops reproduction in early summer avoiding having offspring hatch in autumn when food availability and temperature levels are decreasing (Fischer 1974; Licht 1973; Cuellar and Cuellar 1977; Botte and Angelini 1980). In *Podarcis s. sicula* this refractoriness seems to be mainly endogenously regulated (Angelini *et al.*,1976), but at present only scattered indications are available as to its determinism. We report some evidence that gonads themselves produce, during the refractory period, some principles which result inhibitory for both germ cell maturation and endocrine gonadal activity.

MATERIALS AND METHODS

From 100 adult males and as many females of the lizard *Podarcis s. sicula*, captured during the refractory period (end of July), the gonads were collected to prepare aqueous and lipid extracts. Tissues were homogenized in cold acetone (1 : 10 *w/v*); tissue residues were collected by centrifugation and reextracted twice with cold acetone. The acetone powder, completely free of the solvent, suspended in cold reptilian saline, constituted the *aqueous extract.*

(*) Lavoro eseguito presso l'Istituto di Istologia ed Embriologia e l'Istituto di Zoologia dell'Università di Napoli.

(**) Nella seduta del 12 dicembre 1981.

The combined supernatants, dried under vacuum, gave rise to an oily fluid (7 ml from testes and 5 ml from the ovaries) which represented the *lipidic extract.*

The biological activity of these extracts was tested on winter lizards (obtained in January) whose gonadal activity in both sexes can be stimulated by high temperature (Angelini *et al.*, 1976; Botte *et al.*, 1978). The experimental protocol comprised three animal groups, each composed of 20 adult males and as many females, reared in terraria having a high temperature (30 °C) and natural photoperiod. Food, meal worm larvae and fresh vegetables, were given *ad libitum.* The following treatments were done:

Group 1. Each male received, intraperitoneally twice a week, 0.1 ml of aqueous extract of refractory testes (1 ml of saline contained the acetone powder corresponding to three testes). Each female was injected with the same amount of refractory ovary aqueous extracts.

Group 2. The males were treated twice a week with 0.025 ml of refractory testes oily extract (1 ml of this oil corresponding to 28 testes). The females received the same amount of refractory ovary oily extract (1 ml corresponding to 36 gonads).

Group 3. Half of the lizard of this group were treated with saline; the others were not treated at all. Some mortality (about 15%) was observed in lizards injected with oily extracts.

After 14, 29, and 51 days from the beginning of the treatments, four males and as many females of each group were randomly chosen and sacrificed. In the males hemipenes and phemoral pores development was recorded and then testes and epididymes were excised and fixed in Stieve's fluid for histological examination. Ovaries and oviducts were obtained from females and treated as the male samples.

RESULTS

Males. In the *untreated lizards* high temperature induced spermatogenesis recovery and hemipenes, phemoral pores and epididymis (SSC) development already after 14 days. After 29 days spermatogenesis was very active and many spermatozoa had reached the hypertrophic epididymal lumen. These aspects and SSC development were well evident after 41 days of treatment.

In males receiving testicular *aqueous extracts,* during the first week the spermatogenesis was activated as in the untreated lizard. Starting from 29 days, however, a progressive decrease in sperm production became evident since a limitation in spermatogonial mitoses occurred. The seminiferous tubule diameters, moreover, diminished and oedematous spaces appeared in their walls. Leydig cells, epididymis and other SSC, on the contrary, were developed as in the untreated lizards.

In the animals injected with testicular *lipidic extracts*, after 14 days the spermatogenesis was slowed down and thereafter completely stopped in the last phases. Histological examination showed spermatid degeneration and a decrease in spermiohistogenesis. The Leydig cells reamained undeveloped, as in winter lizards. The same aspect was shown by epididymis and the other SSC.

Females. High temperature induced in the *untreated lizards* the disappearance of ribosome crystalline bodies in the oocytes. This process preludes vitellogenesis (Taddei 19720; Taddei *et al.*, 1973) which, however, was never observed in our animals. The oviducts progressively grow to become completely mature after 51 days.

The ovarian *aqueous extracts* injection had very limited effects; only some delay in oocyte ribosomal crystalline bodies disappearance was observed.

In the lizards administered ovarian *lipidic extracts*, the ovary kept the winter aspect (in the previtellogenic oocytes the crystalline ribosomial bodies persisted).

In some cases several follicles showed the first signs of atresia. The oviduct, moreover, was completely quiescent.

Discussion

Our findings support the hypothesis that the gonads of the lizard, *Podarcis s. sicula*, elaborate, during the postreproductive period (July), some principles that can induce their refractory behaviour. The substances which can be recovered in both the aqueous and the lipid gonadal extracts interfere with both germ cell and SSC development when injected into nonrefractory winter lizards. Their chemical nature, however, is at present unknown since only crude gonadal extracts have been used in our experiments.

Some considerations can be in any case drawn. Testicular *aqueous extracts*, if injected in to males, limit spermatogonial mitoses. This mimics the mammalian "inhibition" action which, by decreasing the FSH release by pituitary, causes a slowing of spermatogonial mitoses (Eshkol and Lunenfeld 1975; Chari 1977; Franchimont *et al.*, 1977; Sethell *et al.*, 1977). More difficult to interpret is the intervention of ovarian aqueous extracts on oocyte development.

In both sexes the lipidic extracts are very active, since they completely prevent the gonadal resumption which is induced by high temperature in winter lizards. These effects could be linked to an inhibition of LH release which in turn abolishes the gonadal sex hormone production. As a consequence the SSCs do not develop and gametogenesis is stopped. Unfortunately there are no detailed indications on sex hormone dependence of gametogenesis in lizards. It has been shown that in adult active males of *Podarcis s. sicula*, estradiol administration is followed by germ cell degeneration and SSC atrophy (Botte and Delrio 1967). The inhibition of spermatids and spermiohistogenesis in male treated with lipidic extracts supports the previously reported hypothesis since in mammals, after

an androgen deprivation, a limitation of germ cell meiosis is observed (Steinberger and Steinberger 1975; Chemes *et al.*, 1979).

In females, the inhibitory effects on gonads could be linked to a mechanism similar to that reported for the iguanid, *Anolis carolinensis*. In this lizard after the breeding period the atresic follicles of the ovary secrete a substance (steroid?) which decreases the sensibility of the organ to gonadotropin (Crews and Licht 1974; Crews 1977, 1979). It is interesting to note that in the ovaries of *Podarcis s. sicula*, treated with the lipidic extracts, an increase of follicular atresia is observed.

In conclusion, our observations, even if preliminary, bring new informations of refractory determinism in temperate zone living lizards and can be the basis for more investigations on this phase of the sexual cycle.

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