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Gaetano Ciarcia, Francesco Angelini, Virgilio Botte

Effects of a chronic treatment with the luteinizing hormone-releasing hormone agonist, buserelin, on the gonads of the lizard Podarcis s. sicula Raf

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Biologia. — *Effects of a chronic treatment with the luteinizing hormone-releasing hormone agonist, buserelin, on the gonads of the lizard* Podarcis s. sicula *Raf.* (*). Nota di GAETANO CIARCIA, FRANCESCO ANGELINI e VIRGILIO BOTTE, presentata (**) dal Corrisp. G. CHIEFFI (***).

RIASSUNTO. — Sono stati studiati gli effetti di un trattamento cronico con un agonista del fattore di rilascio dell'LH,[D–Ser(Bu^t)^6] LHRH(1–9)etilammide (buserelin), sull'apparato riproduttore del lacertide *Podarcis s. sicula.*

Nei maschi la ripresa della gonade è ostacolata dalla somministrazione giornaliera di buserelin. Nei tubuli seminiferi la spermatogenesi è rallentata, molti spermatozoi presentano alterazioni morfologiche; si osservano inoltre alcune strutture citologiche simili a follicoli ovarici. L'epididimo e i caratteri sessuali secondari diventano progressivamente atrofici.

Nelle femmine la somministrazione giornaliera di buserelin inibisce sia la crescita dei follicoli e la vitellogenesi che lo sviluppo dell'ovidutto.

Nei maschi trattati ogni quattro giorni con buserelin si nota invece una marcata stimolazione della spermatogenesi e delle vie genitali.

I dati riportati propongono per l'LHRH agonista un ruolo nei rettili abbastanza simile a quello riportato per i mammiferi.

The investigations by King and Millar (1980) on the comparative aspects of luteinizing hormone-releasing hormone (LHRH) in vertebrates suggest that, in reptilian hypothalamic extracts, this peptide is structurally different from mammalian and amphibian LHRH. Besides these differences, however, this LHRH is biologically active in stimulating the LH release from ovine pituitary cells in culture. Conversely, mammalian LHRH or a potent agonist administered for short time to adult sea turtles *Lepidochelys olivacea* had no measurable effect on immunoreactive plasma level of LH or steroids in either sex (Licht *et. al.*, 1982).

The above data suggest that in reptiles, besides the synthesis of a peculiar LHRH, there also exists a rather homologous hormone-specificity that does not allow them to respond to heterologous hormones. This aspect has prompted us to study this problem also in order to obtain data on the activity of a mammalian LHRH agonist in the lizard, *Podarcis s. sicula.*

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

(**) Nella seduta del 23 giugno 1983.

(***) This paper is affectionately dedicated to Prof. Mario Galgano, on the occasion of his 75th birthday.

Materials and methods

Adult males and females (10–12 g) of the lizard *Podarcis s. sicula* Raf. captured in the neighbourhood of Naples in early March, were used. The animals were housed, with plenty of food, in a special room, where temperature (28 ± 1 °C), relative humidity (78 %) and photoperiod (16 hr daylight) were controlled. These photothermal conditions are the most suitable for inducing early recrudescence of gonadal activity in this species (Angelini *et al.*, 1976). The males (60 specimens) were subdivided into three groups: (1) animals were injected daily s.c. with 0.05 μg of [D-Ser (Bu^t)^6]-LHRH (1–9) ethylamide, (buserelin, Hoe 766), dissolved in 100 μl of reptilian saline; (2) animals were injected every four days with the same amount of buserelin; and (3) control animals were injected daily with 100 μl of reptilian saline. The females (40 specimens) were subdivided into two groups, which received the same treatment as group 1 and 3 of males.

After 5, 10, 20, 30, days of treatment, five males and as many females were killed under ether anesthesia. The gonads and the genital ducts were fixed in Stieve's fluid; serial paraffin sections (7 μm) were stained with Galgano's trichrome stain.

Results

Males. In March the seminiferous tubules of *Podarcis s. sicula* in the field contain all the stages of germ cells, but spermatogonial mitoses are absent and meioses are blocked. The epididymis and secondary sexual characters (SSC) are completely atrophic. Maintenance of the animals at 28 °C and 16 hr daylight induces after 10 days complete recrudescence of spermatogenesis, with intense sperm release and growth of the epididymis and SSC (Figs. 1 and 2). This aspect lasts 20 days more and on the 30th day, spermatogenesis and epididymis show the first signs of regression. The daily treatment with buserelin does not prevent at the beginning (after 5 days) a slight resumption of spermatogenesis and of the epididymis. After 10 days spermiohistogenesis is markedly reduced and, intermingled with germ cells, large elements similar to diplotenic primary oocytes appear (Fig. 3). At this stage many spermatozoa are anomalous, with large and deformed heads (Fig. 4); within spermatids frequent degenerations are observed. Within spermatogonia mitoses continue to occur as usual. The epididymis is completely devoid of secretion. After 20 and 30 days the thickness of the seminiferous tubules is reduced, and few spermatozoa and spermatids are observed (Fig. 5). Spermiation is completely lacking and hence the epididymal lumina are devoid of spermatozoa (Fig. 6). The SSC are still atrophic.

In the lizards treated with buserelin every four days there is a gonadal stimulation similar to that of the controls, but unlike the latter, after 30 days, spermatogenesis and SSC continue to show their maximum activity.

Females. In March, in the field, both the ovary and the oviduct are completely quiescent. In the ovary there are several small follicles and some previtellogenetic oocytes. In the oviduct the glands of the tubal and uterine region appear completely regressed. Maintenance at 28 °C and 16 hr daylight induces, after 20 days, vitellogenesis in the previtellogenetic follicles and growth in the primordial ones. In the oviduct, after 10 days, the uterine and tubal glands show a complete development (Fig. 9). After 30 days the ovary and the oviduct still appear active. The daily treatment with buserelin induces first (after 5 and 10 days) a slight resumption of the oviduct and the ovary, with a development of some small follicles up to the previtellogenetic stage; in the germinative lamina, oogonial mitoses take place as usual (Fig. 8). Vitellogenesis is, however, completely lacking and after 20 days the largest previtellogenetic follicles become atresic (Fig. 7); in the oviduct signs of involution are observed (Fig. 10). After 30 days, numerous previtellogenetic follicles show atresia and the oviduct has re-acquired its typical aspect of winter animals.

DISCUSSION

Our data demostrate that buserelin, a LHRH agonist in mammals, injected in prolonged doses, can induce an inhibitory action on the gonads of the lizard *Podarcis s. sicula.* This action can be compared in many ways to that reported in mammals (Sandow *et al.*, 1980). Conversely, in the chelonian *Lepidochelys olivacea*, short time administration of LHRH or its agonist cannot modify the hematic levels of gonadotropins or sex hormones (Licht *et al.*, 1982). As far as the mode of action of buserelin is concerned, the hypothesis can be formulated that, as in mammals, the alterations of the gonads are the consequence of a diminishing in the number of gonadal LH receptors and/or of a partial decrease in the gonadotropin pituitary production (Sandow *et al.*, 1980). In our experiments, a decrease in androgens, presumably due to a lack of gonadotropins, might be evidenced in treated lizards by the regression of the epididymis and SSC which are testosterone-dependent organs (Dufaure and Gigon, 1975). The block of spermiation and the alterations of sperms might be due to the same cause. Analogous phenomena were observed in lizards treated with ciproterone which blocks the androgen receptors in target organs (Della Corte *et al.*, 1972). Unfortunately, some difficulties in getting enough blood samples from these lizards did not allow direct determination of circulating sex hormones. The mechanisms leading to the formation of the " oocyte-like " structures are unknown. The stimulation of gonadal activity in lizards treated every four days with buserelin is consistent with analogous observations in *Rana catesbeiana* (McCreery *et al.*, 1982) and in mammals where the pulsatile administration of synthetic GnRH re-established pituitary gonadotropin secretion (Belchetz *et al.*, 1978). In female lizards a decrease in ovarian hormone synthesis, following chronic treatment might be evidenced by the absence of vitellogenesis and by the atrophy of the oviduct, whose seasonal development is affected by ovarian

steroids (Botte, 1974). In addition, the persistence in our experiments of spermatogonial and oogonial mitoses proves some FSH sensitivity in the gonads of treated animals, since a FSH-like hormone in reptiles stimulates the proliferation of these cells (Jones *et al.*, 1976). Lastly, it cannot be excluded that the effects observed are, at least in part, linked to a local buserelin action. Our preliminary investigations show that at the end of the breeding period in *Podarcis s. sicula* gonads, substances are produced that immunocross-react with the antibodies of mammalian hypothalamic LHRH and of an agonist similar to the testicular mammalian LHRH (Angelini *et al.*, unpublished data). Moreover, it is well known that, in mammals, LHRH has local regulatory functions on sex hormone synthesis in both sexes (Magoffin and Erickson, 1982; Sharpe and Cooper, 1982). The present study suggests that in reptiles the mammalian LHRH agonist acts, at least in chronic treatments, as in mammals. The use of LHRH agonist may be an important aid in elucidating the mechanisms of the endocrine control of reproduction in lower vertebrates.

References

Angelini F., Picariello O. and Botte V. (1976) - « Boll. Zool. », *43*, 111-123.

Belchetz P. E., Plant M. T., Nakai Y. Keogh E. J. and Knobil E. (1978) - « Science », *202*, 631-632.

Botte V. (1974) - « Monitore Zool. ital. », *8*, 47-54.

Della Corte F., Angelini F., Varano L. and Cavagnuolo A. (1972) - « Atti Soc. Pelor. Sci. fis. mat. nat. », *19*, 17-33.

Dufaure J. P. and Gigon A. (1975) - « Gen. Comp. Endocrinol. », *25*, 112-120.

Jones R. E., Takarz R. R., La Greek F. T. and Fitzerald K. T. (1976) - « Gen. Comp. Endocrinol. », *30*, 101-116.

King A. J. and Millar R. P. (1980) - « Endocrinology », *106*, 707-717.

Licht P., Owens D. W., Cliffton K. and Penaflores C. (1982) - « Gen. Comp. Endocrinol. », *48*, 247-253.

Magoffin D. A. and Erickson G. F. (1982) - « Mol. Cell. Endocrinol. », *27*, 191-198.

McCreery B. R., Licht P., Barnes R., Rivier J. E. and Vale W. W. (1982) - « Gen. Comp. Endocrinol. », *46*, 511-520.

Sandow J., Rechenberg W., Jerzabek G., Engelbart K., Kuhl H. and Fraser H. (1980) - « Acta Endocrinol. », *94*, 489-497.

Sharpe R. M. and Cooper I. (1982) - « Mol. Cell. Endocrinol. », *27*, 199-211.

EXPLANATIONS OF PLATE I

Fig. 1. – Cross section of seminiferous tubules with active spermatogenesis in a control animal kept for 10 days at 28 °C. × 110.

Fig. 2. – Cross section of epididymal tubules, full of spermatozoa and secretion, of the same animal as Fig. 1. × 200.

Fig. 3. – Cross section of a seminiferous tubule of an animal treated for 10 days with buserelin. Note the " oocyte–like " structures. × 200. Insert: higher magnification of an " oocyte–like " structure. × 320.

Fig. 4. – Portion of a seminiferous tubule of an animal treated for 10 days with buserelin. Note the anomalous aspect of several spermatozoa. × 240.

Fig. 5. – Cross section of a seminiferous tubule of an animal treated for 20 days with buserelin. The sperm number is reduced. × 200.

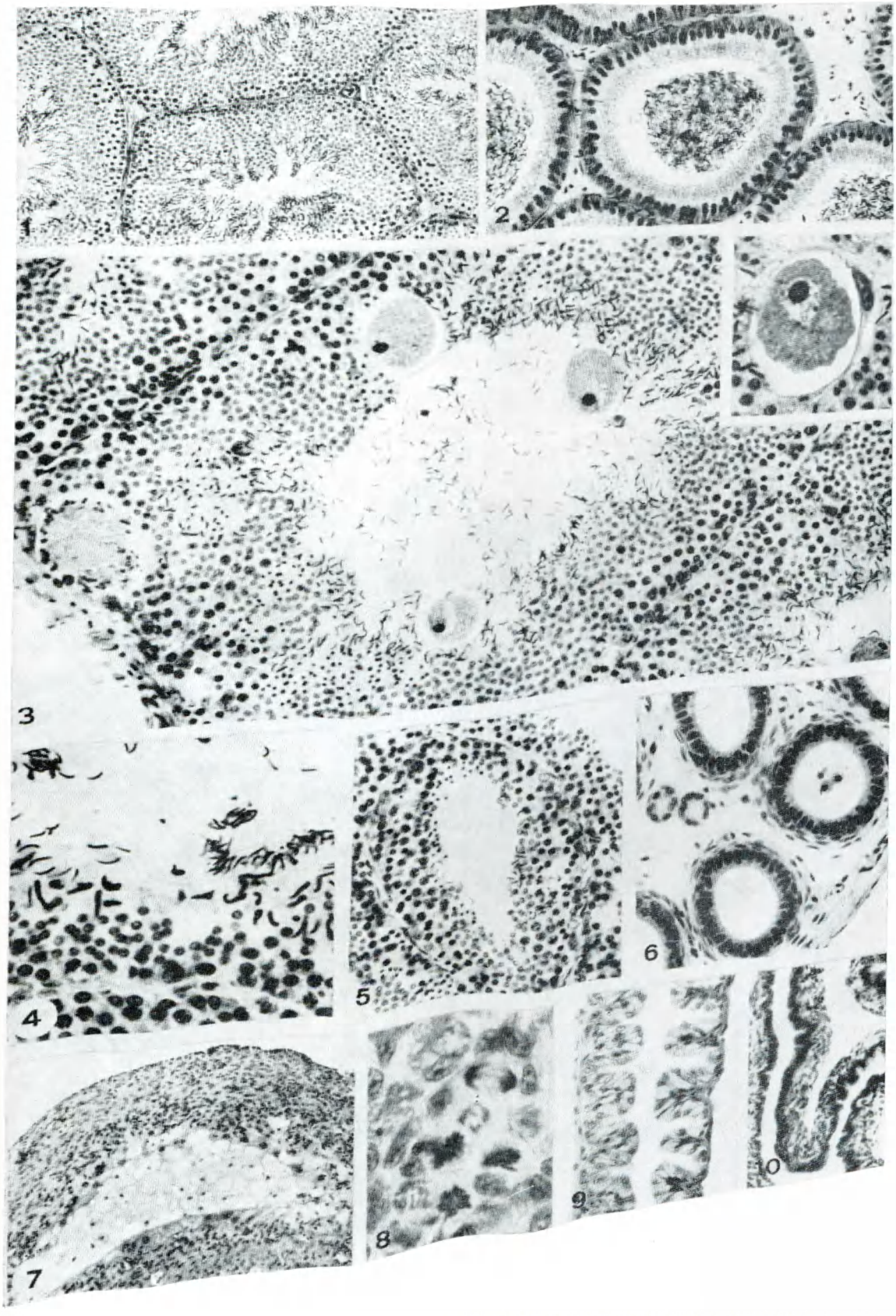
Fig. 6. – Involuted epididymis of the same animal as Fig. 5. × 200.

Fig. 7. – Atresic follicle of an animal treated for 20 days with buserelin. × 160.

Fig. 8. – Oogonial mitoses of a lizard treated for 10 days with buserelin. × 850.

Fig. 9. – Developed tubal tract of the oviduct of a control animal kept for 20 days at 28 °C. × 180.

Fig. 10. – Involuted tubal tract in the oviduct of a lizard treated for 20 days with buserelin. × 180.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