Arginine-vasotocin and gonadal activity in the lizard, *Podarcis s. sicula* Raf.

G. Ciarcia, F. Angelini, O. Picariello and V. Botte

Istituto di Istologia ed Embriologia and Istituto e Museo di Zoologia, Università di Napoli, Via Mezzocannone, 8, I-80134 Napoli (Italy), June 21, 1982

Summary. Arginine-vasotocine (AVT) injected into male adult lizards, *Podarcis s. sicula* Raf., inhibits the last phases of spermatogenesis and the endocrine activity of gonads. In female lizards the substance induces an early interruption of egg deposition.

In mammals, arginine-vasotocin (AVT) is secreted by the epiphysis but is not present in the neurohypophysis[1-5]. It is assumed to influence gonadal function through an inhibition of LH-RH output[6-8] and/or of LH release by the pituitary gland[5-10]. AVT administration to prepubertal mice, moreover, prevents the development of the gonads and of the sexual secondary characters (SSC), also in those animals which received PMS or hCG injections[5,9,10].

In reptiles, AVT is secreted by the neurohypophysis and influences oviduct motility[11-16]. Recently its synthesis by the epiphysis was demonstrated[17]; however, the function of AVT from this source is not known. In these vertebrates, the epiphysis being involved in the mechanisms of photoperiodism action on the genital apparatus[18-21], it was of interest to study the influence of AVT on gonadal functions.

Materials and methods. Adult lizards, *Podarcis s. sicula* Raf., recently captured, were obtained in mid-April, just prior to the seasonal gonadal recrudescence and breeding period[22-24]. They were divided in 2 groups and mantained in open terraria with plenty of food. The animals of the 1st group (17 males and 19 females) received 0.1 ml saline containing 1 µg of AVT (Vasotocin-Sigma V 4552) i.p. every other day. The lizards of the 2nd group (15 males and 15 females) were injected with the solvent only. The mortality rate was close to zero. 15, 30, and 45 days after the beginning of the experiment, 5 males and 5 females from each group were weighed and then sacrificed. Weights of gonads and oviducts were determined. Gonads, epididymis and oviducts were fixed in Stieve's fluid and processed for histological examination.

Results and discussion. AVT treatment does not provoke significant differences in the body weights, in the weights of gonads and oviducts, if compared with the values of the controls in the same periods. The histological examination of the testes of the controls shows an active spermatogenesis throughout the experiment, similarly to what is observed in free-living lizards[25]. Both spermatogonial mitoses and all the phases of meiosis are quite evident. The spermatozoa output is abundant and constant (fig. 1). Spermatozoa are released into the lumen of the seminiferous tubules and are found in abundance in the ducts of the epididymis, that

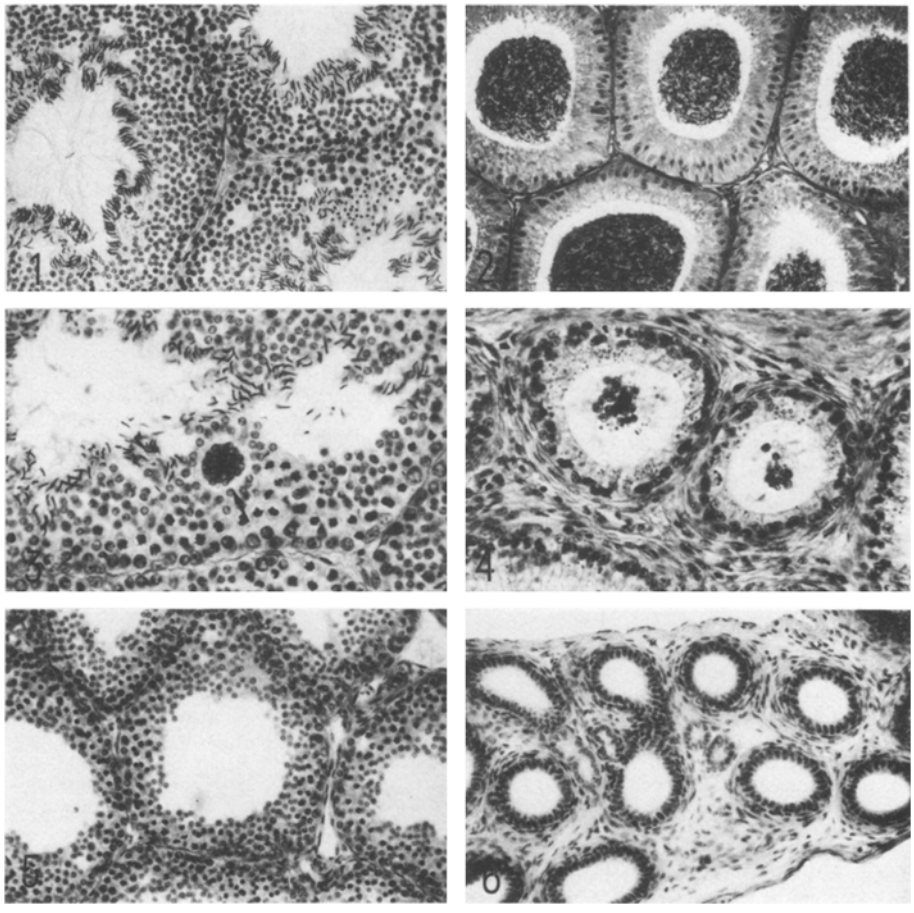

Figure 1. Cross section of *Podarcis s. sicula* Raf. testis treated for 45 days with reptile saline. Spermatogenesis is very active and Leydig cells are well developed. × 165.

Figure 2. Section of the epididymis of the same lizard as figure 1. Note that the epithelial layer is well developed and that lumina are filled with sperms. × 165.

Figure 3. Section of a lizard testis treated for 15 days with AVT. Spermiohistogenesis is partially impaired. The arrow points to a clump of degenerating spermatids. × 265.

Figure 4. Section of the epididymis of the same lizard as figure 3. Note that the epithelial layer is reduced. In the lumina clumps of degenerating spermatids and spermatocytes II can be observed. × 265.

Figure 5. Section of a testis of a lizard treated for 45 days with AVT. The postmeiotic phases of spermatogenesis are completely lacking. × 165.

Figure 6. Section of the epididymis of the same lizard as figure 5. A complete regression of the tubules can be observed. Moreover, the lumina are devoid of sperms and secretion. × 165.

Ovoposition trend in females, *Podarcis s. sicula* Raf., treated with AVT during the breeding period

Period No.	May 1-15 Animals	Eggs	May 16-31 Animals	Eggs	June 1-15 Animals	Eggs	Total eggs
Controls	15	39	10	28	5	25	92
Treated*	19	38	14	56	9	0	94

*Total AVT doses at the end of each period were respectively μg 8, 16 and 23.

appears to be hypertrophic and actively secreting (fig. 2). In treated animals, already after 15 days, and more clearly after 30 days, while spermatogonial mitoses and spermatocytes I in meiosis show an aspect similar to those of the controls, several degenerative phenomena involve spermatocytes II and spermatids. Moreover, a drastic decrease in the spermatozoa count is observed (fig. 3). The epididymis drastically regresses and shows a decrease in height of the epithelial cells and in their secretion. In its ducts, degenerating germ cells are found, whereas spermatozoa are completely lacking (fig. 4). After 45 days of treatment spermatogonial mitoses and meioses still persist, but only few spermatocytes II and some spermatids are produced. Spermatozoa, instead, are completely lacking (fig. 5). The epididymis regresses and appears empty (fig. 6).

In females no remarkable differences are observed in the ovarian histology between control and treated lizards. The latter, however, stop ovoposition earlier than the controls (table) and have a more developed muscular layer in the oviducts.

The data reported show that AVT, administered to male and female adult lizards, *Podarcis s. sicula* Raf., does not modify either body weight or the weight of gonads and oviducts, if compared with the controls, even after 45 days of treatment. In the male, AVT has antigonadal action, by progressively inhibiting spermiohistogenesis and sperm release. At the same time an epididymal atrophy takes place. These effects might depend on an inhibition of LH release and, consequently, on a decrease in androgen output, as in mammals, where a similar situation is found.

The persistence of spermatogonial mitoses in treated lizards might depend on a FSH-like hormone, whose release would not be influenced by AVT. This is consistent with what was demonstrated in many mammals, where spermatogonial mitoses are under the FSH control[26]. This hypothesis, however, is in contrast with what was so far suggested by some authors e.g., that in this species a single FSH-like gonadotropin controls both testis endocrine activity and spermatogenesis[27-29]. Androgen decrease, following a possible LH decrease, might be, therefore, responsible both for spermiohistogenesis break down and for epididymal atrophy. In reptiles, whereas androgen-dependence of the epididymis and of the SSC is documented[30,31], their implication in the regulation of the various stages of spermatogenesis is still scarcely proven[32].

The interference of AVT with the egg production of female lizards could be interpreted as an inhibition of late vitellogenetic processes, but this effect might also be due to the presence of treated males, whose sexual activity was remarkably impaired by AVT injections. In fact, these males show reduced hemipenes, as well as an inhibition of gonads and epididymis. As shown in the lizard, *Anolis carolinensis*, active males with well-developed hemipenes are necessary for the occurrence of a normal vitellogenesis in females[33,34]. The hypertrophy of the muscular layer of the oviduct might depend on the strong AVT effect on this tissue[15,16].

Our data support the hypothesis that in lizards, as in mammals, AVT might have a strong influence on gonadal function. When secreted by the epiphysis, it might be a mediator of photoperiod stimulations on the reproductive apparatus of reptiles.

1 Milcu, S.M., Pavel, S., and Neacsu, C., Endocrinology *72* (1963) 563.
2 Pavel, S., Demitru, I., Klepsh, J., and Dorcescu, M., Neuroendocrinology *13* (1973) 41.
3 Legros, J., Louis, F., Demoulin, A., and Franchimont, P., J. Endocr. *69* (1976) 289.
4 Pévet, P., Ebels, I., Swaab, D.F., Mud, M.T., and Arimura, A., Cell Tissue Res. *206* (1980) 341.
5 Moszkowska, A., and Ebels, I., Experientia *24* (1968) 610.
6 Yamashita, E., Niemo. M., and Yamashita, Er., J. Endocr. *81* (1979) 103.
7 Yamashita, K., Niemo, M., and Yamashita, Er., J. Endocr. *84* (1980) 449.
8 Niemo, M., Yamashita, Er., Koba, H., Iomori, M., and Yamashita, K., IRCS med. Sci. *8* (1980) 458.
9 Vaughan, M.K., Vaughan, G.M., and Klein, D.C., Science *186* (1974) 938.
10 Vaughan, M.K., Vaughan, G.M., and Reiter, R.J., Experientia *31* (1975) 862.
11 Sawyer, W.S., Am. Zool. *17* (1977) 727.
12 Pickering, E.T., J. Endocr. *39* (1967) 285.
13 Acher, R.J., Chauvet, J., and Chauvet, M.T., Gen. comp. Endocr. *19* (1972) 345.
14 Acher, R.J., in: Handbook of physiology, vol. 4, p. 119. Eds E. Knobil and W.H. Sawyer. American Physiological Society, Washington, D.C. 1974.
15 La Pointe, J.L., J. Endocr. *43* (1969) 197.
16 Guillette, L.J., and Jones, R.E., J. exp. Zool. *212* (1980) 147.
17 Vivien-Roels, B., Guerne, J.M., Holder, F.C., and Schroeder, D.M., in: Brain research, vol. 52, p. 459. Eds J.A. Kappers and P. Pévet. Elsevier/North Holland, Amsterdam 1979.
18 Stebbins, R.C., and Cohen, M.W., Copeia *4* (1973) 662.
19 Levey, I.L., J. exp. Zool. *185* (1973) 169.
20 Licht, P., and Pearson, A.K., Copeia *1* (1970) 171.
21 Angelini, F., Botte, V., and Boschi, F., Monitore Zool. ital. *14* (1980) 27.
22 Filosa, S., Monitore Zool. ital. *7* (1973) 151.
23 Botte, V., Angelini, F., Picariello, O., and Molino, R., Monitore Zool. ital. *10* (1976) 119.
24 Angelini, F., Picariello, O., and Botte, V., Boll. Zool. *43* (1976) 111.
25 Galgano, M., and D'Amore, C., Boll. Zool. 27 (1960) 1.
26 Hochereau-de-Reviers, M.T., in: Reproductive process and contraception, p. 307. Eds W. Kennet and B. McKerns. Plenum Publishing Corporation, 1981.
27 Licht, P., Gen. comp. Endocr. *22* (1974) 463.
28 Licht, P., Papkoff, H., Farmer, S.W., Muller, C., Tsui, H.W., and Crews, D., Rec. Prog. Horm. Res. *33* (1977) 169.
29 Licht, P., Farmer, S.W., Bona Gallo, A., and Papkoff, H., Gen. comp. Endocr. *39* (1979) 34.
30 Della Corte, F., Angelini, F., Varano, L., and Cavagnuolo, A., Atti Soc. pelorit. Sci. fis. mat. nat. *19* (1973) 17.
31 Dufaure, J.P., and Gigon, A., Gen. comp. Endocr. *25* (1975) 112.
32 Callard, J.P., Callard, G.V., Lance, V., Bolaffi, J.L., and Rosset, J.S., Biol. Reprod. *18* (1978) 16.
33 Crews, D., Rosenblatt, J.S., and Lehrman, D.S., Endocrinology *94* (1974) 541.
34 Crews, D., in: Endocrine control of sexual behavior, p. 167. Ed. C. Beyer. Raven Press, New York 1979.

0014-4754/83/060615-02$1.50 + 0.20/0