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Effects of Gonadotrophin-Releasing Hormone Variants on Reproductive Organs and Plasma Testosterone in the Male Lizard, *Podarcis s. sicula*

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Abstract

The effects of various gonadotrophin-releasing hormone (GnRH) forms (mammalian GnRH (mGnRH), chicken I GnRH (cGnRH-I), chicken II GnRH (cGnRH-II) and salmon GnRH (sGnRH)) on the genital apparatus and plasma testosterone level in the male lizard, *Podarcis s. sicula*, have been investigated.

In short duration experiments (20 min to 76 h) GnRH forms did not affect testicular and epididymal morphology. A single dose (0.05 μg) of mGnRH, cGnRH-II and sGnRH, however, induced a rise in plasma testosterone after 20 to 40 min. Variable results were obtained in the animals given GnRH variants every 12 h for 3 days since mGnRH and cGnRH-I caused a decrease of circulating hormone; cGnRH-II and sGnRH a slight increase.

Daily peptide administration, for 15 to 30 days, caused severe inhibition of both testicular and epididymal activity and a significant drop of circulating testosterone.

In *Podarcis s. sicula*, species specificity of pituitary sensitivity to GnRH variants appeared to be low. On the other hand, this gland seemed to show some desensitization after chronic peptide administration.

In reptilian brain there are several gonadotrophin-releasing hormone (GnRH) variants, none of which are identical to mammalian GnRH (mGnRH) (1, 2). Apart from some novel forms of unknown structure, salmon GnRH (sGnRH), chicken I GnRH (cGnRH-I) and chicken II GnRH (cGnRH-II) have been identified. These findings confirm that there is a wide molecular heterogeneity of these peptides in vertebrate tissues (1–3).

Three GnRH variants have been obtained from the brain of the lizard *Podarcis s. sicula*. Two of these showed retention time on HPLC identical to those of sGnRH and cGnRH-I, respectively. These affinities were confirmed by immunological studies and by the *in vitro* luteinizing hormone- (LH-) releasing activity on chicken pituitary. The third variant was a novel form of GnRH (4). Besides brain and hypothalamus, gonads, liver and small intestine also contain GnRH-like immunoreactive substances whose amount fluctuates during the year (5).

Data on the functions of GnRHs in *Podarcis s. sicula* are, at present, very scanty. In the adult male, a mGnRH agonist, buserelin, blocks the spermatogenetic recrudescence when administered daily, whereas it tends to prolong the period of maximal testicular activity if injected every 4 days (6). Such effects, which are consistent with those obtained in mammals (7), support a wide responsiveness of lizard pituitary to GnRHs. To test this hypothesis, and to obtain information on the biological activity of different GnRH forms in this lizard, we evaluated in adult males the influence of GnRH variants on genital apparatus and plasma testosterone levels.

Results

In the short duration experiments (20 min to 76 h), GnRH variants did not affect testicular or epididymal morphology. The latter organ is a typical androgen-dependent secondary sexual character. In the gonads of both control and treated lizards, spermatogenesis was active and sperms were present in seminiferous tubules. The epididymis was well developed and lined with columnar secretory epithelium.

A single dose (0.05 μg) of mGnRH, cGnRH-II and sGnRH induced, 20 to 80 min later, a significant increase of plasma testosterone levels ($P < 0.05$). mGnRH and cGnRH-II were more active than sGnRH ($P < 0.05$). A plasma testosterone increase was also observed in lizards injected with cGnRH-I, but it was not statistically significant (Fig. 1).

Variable results were obtained in the animals given GnRH variants every 12 h for 3 days. mGnRH caused a slight decrease of plasma testosterone whereas cGnRH-I brought about a clear

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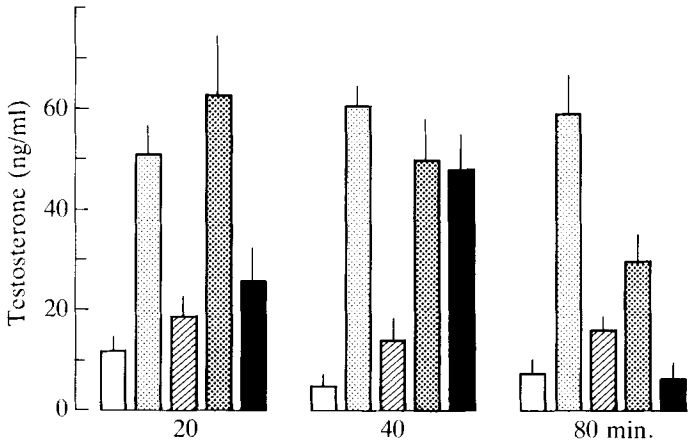

FIG. 1. Effects of a single dose (0.05 μg) of various GnRH forms on plasma levels of testosterone in the male lizard, *Podarcis s. sicula*. □—controls (injected with solvent); ▨—mGnRH; ▨—cGnRH-I; ▨—cGnRH-II; ■—sGnRH. See Results for significance levels.

decrease of the hormone ($P < 0.01$). cGnRH-II and sGnRH, on the contrary, induced an increase in circulating testosterone titres; this effect, however, was statistically significant ($P < 0.05$) only in the lizards killed 28 h after the onset of treatment with cGnRH-II (two doses) (Fig. 2).

When injected daily for 15 to 30 days all the peptides caused an inhibition of testicular and epididymal activity. The gonads and epididymes of controls were active throughout the experiment. In treated male lizards, spermatogenesis was blocked; a few sperms were still present in the seminiferous tubules. In the epididymis, signs of atrophy became evident and the lining epithelium appeared non-secretory. Sperms were absent from the lumen.

In controls, the plasma testosterone levels decreased throughout the experiment. In lizards treated with various GnRH forms, however, a more consistent drop of circulating hormone was observed ($P < 0.05$). This effect, after 30 days of treatment, was more intense in the groups injected with sGnRH ($P < 0.01$) (Fig. 3).

Discussion

Data on the effects of *in vivo* administration of GnRH in reptiles are conflicting (1, 3, 8). Low doses of mGnRH stimulated a rise in

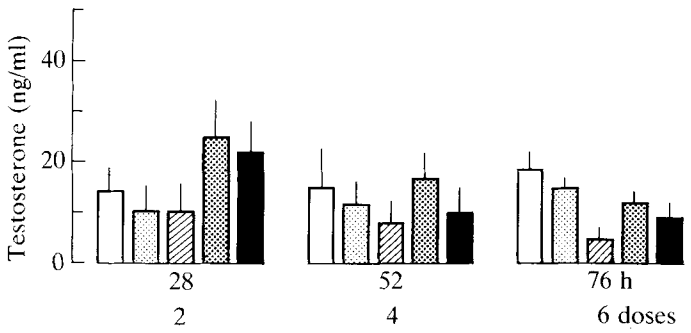

FIG. 2. Effects of various GnRH forms (0.05 μg given every 12 h) on plasma levels of testosterone in the male lizard, *Podarcis s. sicula*. □—controls (injected with solvent); ▨—mGnRH; ▨—cGnRH-I; ▨—cGnRH-II; ■—sGnRH.

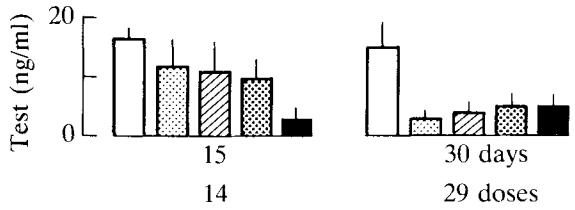

FIG. 3. Effects of various GnRH forms (0.05 μg given every 2 days) on plasma levels of testosterone in the male lizard, *Podarcis s. sicula*. □—controls (injected with solvent); ▨—mGnRH; ▨—cGnRH-I; ▨—cGnRH-II; ■—sGnRH.

plasma LH and progesterone in the female turtle *Chrysemis picta* (9). This effect, however, was not confirmed in the female by other authors who found, on the other hand, that males of the same species were responsive to mGnRH (10). mGnRH, or its agonists, failed to alter plasma LH and gonadal steroids in male turtles *Chelonia mydas* and *Lepidochelys olivacea* and in males and females of the turtle *Sternotherus odoratus* and cobra *Naja naja* (8, 11). In the latter two species cGnRH-I was also without any effect (10). mGnRH stimulated plasma testosterone secretion in the male alligator (12). In the female iguana, *Iguana iguana*, cGnRH-II caused a rise in plasma oestradiol and elicited sexual behaviour in cohabiting males (13). mGnRH induced sexual behaviour in the female lizard *Anolis carolinensis* (14), but the male was unresponsive to this peptide (3).

Our tests indicate that the male lizard, *Podarcis s. sicula*, shows a wide sensitivity to GnRHs since a single dose (0.05 μg) of mGnRH, cGnRH-II and sGnRH induces, after 20 to 40 min, a significant increase of circulating testosterone. These effects are still detectable in animals treated for 2 days with cGnRH-II and sGnRH.

The above-reported discrepancies have been ascribed to species specificity in pituitary sensitivity to GnRHs in reptiles. Recent observations, however, seem to invalidate this contention. It has been shown, in fact, that in some species GnRH is inactive when injected *in vivo*, but induces LH release when tested on superfused pituitaries *in vitro* (10, 15–17). Many factors can interfere with the *in vivo* procedures and can therefore produce false negative results (3).

As previously reported, sGnRH and cGnRH-I are present in *Podarcis s. sicula* brain (4). The biological effect of sGnRH described in this paper supports the notion that the peptide is implicated in the regulation of the reproductive processes. An analogous role, in our opinion, could be played by cGnRH-I. Its low activity, when injected *in vivo*, could depend on rapid degradation or clearance. These processes, in fact, may account for a relatively poor activity *in vivo* of some GnRH variants (18). Alternatively, cGnRH-I could play roles in the CNS unrelated to regulation of pituitary gonadotrophin release. In rats, for example, GnRH has been found to affect reproductive behaviour (19).

The relatively high activity of cGnRH-II and mGnRH in *Podarcis s. sicula* confirms that native molecules are not necessarily the most potent of the naturally-occurring forms (17). It is interesting to note, however, that cGnRH-II is present in many of the reptilian species so far examined, but it seems to be absent from *Podarcis s. sicula* brain (1, 4).

The inhibition of testicular and epididymal activity in lizards receiving daily injections of various GnRH confirms previous

observations performed on the same species (6). These effects suggest some pituitary desensitization. The phenomenon has been observed in several vertebrates (18) and, at least in the rat, it depends on 'down-regulation' of GnRH pituitary receptors. It is doubtful if a similar mechanism operates in the pituitary gland of non-mammalian vertebrates (20). Pituitary desensitization to GnRH, moreover, is not a general property of lower vertebrate pituitary; it has not been found in the pituitary gland of the bullfrog *Rana catesbeiana* continuously perfused with mGnRH (21).

In mammals, GnRH-like substances are also produced in the gonads and in several organs of the reproductive apparatus (20, 22–24). In the gonads they seem to intervene in the regulation of sex hormone biosynthesis, probably acting through either paracrine or autocrine mechanisms (25, 26). Such local action has also been proposed in some lower vertebrates (amphibian testis) (27, 28). It cannot be excluded that the results obtained in our experiments depend, in part, on a direct influence of peptides on the gonads. Indeed, in *Podarcis s. sicula* testis, substances cross-reacting with some GnRH antisera have been found, but their chemical nature and function is still unknown (5).

Materials and Methods

Animals

Adult male lizards, *Podarcis s. sicula* Raf, were obtained from the outskirts of Naples during spring when their genital apparatus is active. The animals were housed in terraria and fed on meal worms and fresh vegetables. The terraria were exposed to the outside natural photothermal regime which sustains gonadal activity (29).

Some lizards were killed soon after capture and before the beginning of the treatments to evaluate the developmental stage of the genital apparatus and the plasma androgen levels.

GnRHs

The following GnRH forms were tested: mGnRH (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2); cGnRH-I (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Gln-Pro-Gly-NH_2); cGnRH-II (pGlu-His-Trp-Ser-His-Gly-Trp-Tyr-Pro-Gly-NH_2); sGnRH (pGlu-His-Trp-Ser-Tyr-Gly-Trp-Leu-Pro-Gly-NH_2). These peptides were a gift from Dr R. C. deL. Milton and Dr J. A. King, University of Cape Town, South Africa. The hormones were dissolved in 0.05 N acetic acid in saline (0.5 μg/ml), divided into small aliquots and kept at −30 °C. Immediately before use, aliquots of peptide solution were thawed and diluted 1:10 with 0.05 N acetic acid in saline.

Experimental procedures

Three experiments were conducted. In the first, groups of 12 lizards were each injected ip with 0.1 ml (0.05 μg) of the various peptide solutions and were killed (four animals per group) 20, 40 and 80 min later. In the second experiment, groups of 12 lizards were each injected with the same amount of peptide every 12 h. Four specimens from each group were killed 28, 52 and 76 h after the beginning of hormone administration. In the third experiment, groups of ten lizards received the same amounts of peptides administered every 24 h; the animals were killed (five from each group) 15 and 30 days later. Control lizards, receiving the vehicle only, were included in all the experiments.

Blood was obtained from lizards through a heparinized capillary inserted into the heart. Blood samples were centrifuged to generate plasma which was kept at −20 °C. At dissection, testes and epididymes were removed and used to prepare histological sections according to the usual procedures.

Testosterone determination

Testosterone in plasma samples was determined by RIA as previously reported (30). The testosterone antibody cross-reacted (>7.0%) with dihydrotestosterone; the two hormones were not separated. The assay sensitivity was 7 pg (intraassay variability 7%; interassay variability 13%). All data were analysed using ANOVA followed by the Duncan's test.

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