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Comparative Effects of Mammalian Growth Hormone on Three Species of Anuran Amphibia Before Metamorphosis

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Different results have been obtained by administration of porcine growth hormone to three different species of spanish anuran amphibia tadpoles (*Rana iberica, Rana ridibunda* and *Discoglossus pictus). Discoglossus pictus* does not show response to 5-50 μg doses of STH porcine injected every-other-day for a period of two weeks.

Rana ridibunda shows significative response to administration STH (5 μg) in three alternate injections. *Rana iberica* shows positive response to the administration of three and five alternate injections of STH porcine (20 and 50 μg/injection respectively). This shows that STH porcine has activity on some anuran amphibia species before metamorphosis. A discussion on which may be the factors that regulate normal growth in amphibia before metamorphosis is given in this paper.

Pituitary gland is thought to be necessary for the normal growth of anuran tadpoles since the works of ALLEN (1) and SMITH (21). Hormonal regulation of growth is being widely studied at present.

It has been suggested recently that not only growth hormone or somatotropine (STH) but also, and above all prolactin, are responsible of growth in amphibia (4, 11, 17).

Gigantisme (19, 20) has been observed after administration of growth hormone (STH) to hypophysectomized *Alytes obstetricans tadpoles*. According to ENEMAR *et al.* (9), in *Rana temporaria*, STH is the most effective hormone in stimulating growth before metamorphosis.

Different results to the above mentioned have been achieved by other investigators at the time of studying the effects of either hormone, STH and prolactin, on other species of amphibia. BERMAN *et al.* (2) have shown that prolactin is the main growth facthor in *Rana catesbeiana* tadpoles. BROWN and FRYE (5) assure that before metamorphosis prolactin has stronger effect as stimulant of growth than STH in *Rana pipiens*. ZIPSER *et al.* (23) came to the same conclusion studying the effects of this hormone on *Bufo marinus* and *Bufo boreas*. BROWN and FRYE (6) infer that administration of STH porcine does not stimulate growth in amphibia before metamorphosis and attribute the

positive response found by other investigators to contamination with prolactin.

HUNT and JACOBSON (13) studied the action of STH and prolactin on the brain cells of *Rana pipiens* tadpoles and found that with growth hormone brain DNA increased only during the period of injection. Prolactin, nevertheles, had very little effect during this period, DNA accumulated at high rate during the first two weeks after injection but accumulation decreased after this period.

Considering the above mentioned contradictory results, we determined to analyze comparatively the activity of growth hormone (STH porcine) on three different species of anuran amphibia, before and after metamorphosis, as well as the activity of prolactin on these species.

In the present work we give the results we obtained after administration of STH porcine to *Rana ridibunda, Rana iberica* and *Discoglossus pictus*. We analyze the changes in growth before metamorphosis and, in the case of *Rana iberica*, forestating results obtained that will appear in a further paper, the changes of protein amount and DNA of the animals treated with STH in respect to their controls.

Materials and Methods

We have used three different amphibia: *Rana ridibunda, Rana iberica* and *Discoglossus pictus*. Tadpoles were treated before metamorphosis at different stages of larval growth. They were injected with porcine growth hormone (1 U.I./mg, Calbiochem).

Discoglossus pictus tadpoler were brought from Segovia or from the surroundings to Madrid during Spring 1971. They were kept in a dilute Holtfreter solution (10 %) prepared every-other-day from an initial concentrated solution maintained at low temperature (8° C). The larvae were fed on boiled spinach and kept at room temperature (21 ± 2° C).

The dosis of growth hormone (STH) administered per injection was 5, 10, 20 or 50 μg in 2 μl distilled water. Tadpoles received three injections in some experiments and five in others. Controls were injected 2 μl distilled water at the same time. Larval stages ranged between 32 and 38 of MANNELLI and MARGARITORA's table for *Rana esculenta* (15) and initial lengths ranged between 16 and 23 mm.

The adults were sent to our laboratory from Tarragona and Segovia. Females were induced to ovulate by administration of homoplastic hypophysis extracts. *Rana ridibunda* tadpoles were reared in our laboratory through experimental fertilization. The embryos developed in a Holtfreter saline solution (10 %), at room temperature and were fed on boiled spinach too. The medium was changed every-other-day. In these conditions the tadpoles reached the appropriate stage in 30 days approximately.

Tadpoles were injected at larval stages 30 and 31 of MANNELLI and MARGARITORA's table (15). The total legth of the animals ranged between 9 and 11 mm in the first experiment and between 11 and 15 mm in the second experiment. Controls of the same size and growth stage were selected for both experiments.

The only dosis administered was 5 μg STH in 2 μl distilled water. Controls received, as above, 2 μl distilled water. The animals were injected every three days and the total number of injections was three per animal.

Rana iberica tadpoles were sent from León during the month of May. Larval stages ranged between 35 and 38 of MANNELLI and MARGARITORA's table (15) and their length was 30-35 mm in the first experiment. The animals received an alternate injection (20 μg) during three days. In the second experiment we used animals ranging between 38-44 and lengths between 32-35 mm. The animals were injected daily (50 μg) for five days. Controls of the same length and larval stage were selected for both experiments.

Fig. 1. *Discoglossus pictus tadpoles (right), treated with STH porcine (5 injections, 20 μg/inject.) compared to the control (left) injected with an equal volume of distilled water (5 injections, 2 μl each).*

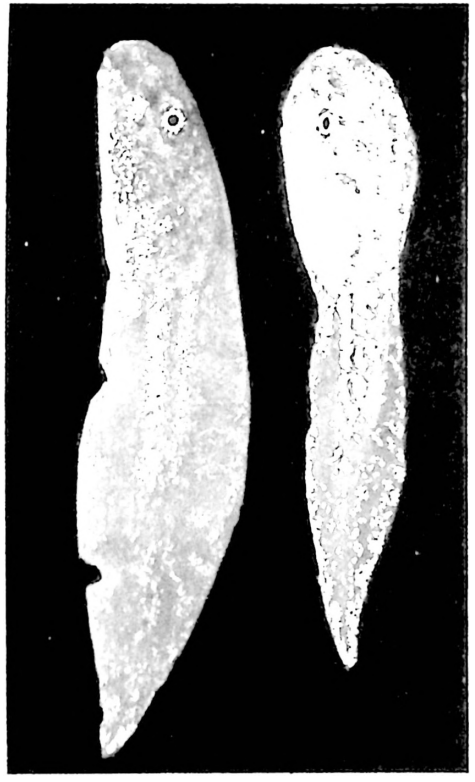

Fig. 2. Rana ridibunda *tadpoles (left), treated with porcine STH (3 injections, 5 μg/inject.) compared to the control (rigth) injected with an equal volume of distilled water (3 injections, 2 μl each).*

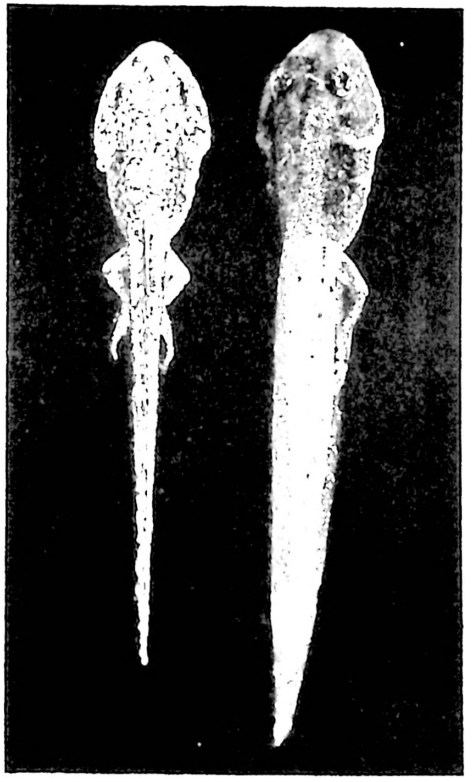

Fig. 3. Rana liberica *tadpoles (rigth), treated with STH porcine (3 injections, 20 μg/inject.) compared to the controls (left), injected with an equal volume of distilled water (3 injections, 2 μl each).*

Tadpoles were anesthetized with ethylic ether previously diluted in Holtfreter solution (10 %). The animals recovered almost immediately and regained their natural vitality few seconds after injection.

Length measurements were taken every two or three days according to the case. The animals were individually kept in Petri dishes.

The injections were performed intraperitoneally by means of HAMILTON micro syringes (50 μl) to which we inserted a thin glass capillar.

Statistical comparisons were made with the percentages of the initial measurement increments. Arithmetic mean and standard error were also estimated.

Student's «t» test of the varied groups in respect to their controls were carried out.

A significant difference is indicated by a «p» value of 0.05 or less.

Results

Table I shows the results obtained by administration of porcine growth hormone to *Discoglossus pictus* tadpoles. As may be deduced from this table, *Discoglossus pictus* tadpoles, after five injections STH (5 μg/injection), undergo an increment in growth, expressed in % of the initial total length increment, smaller than that of the control animals. A possible growth inhibition own to STH has not been considered. With five injections of 10 μg each the increment in growth is bigger than in the control animals but the differences are not significative. Finally, we observed that with 20 μg and 50 μg STH the increment in total growth, expressed in % on the initial total length increment, is also slightly smaller than in the control animals. *Discoglussus pictus* does not show positive response to STH porcine administration. The differences in final length after administration of different doses of hormone are not significative (Fig. 1).

In *Rana ridibunda*, nevertheless, administration of 15 μg STH in only three injections (5 μg/injection), was sufficient to produce, in respect to the control animals, a significative increment in the total initial length. In both groups of experiments growth increment, in respect to the control animals, is significative ($P < 0.01$; $P < 0.02$). In the second experiment the initial lengths and larval stages were higher. This could explain, perhaps, the different results obtained in the two experiments. Significative body length and broadness increments have not been found (Table II, Fig. 2).

Finally, in *Rana iberica*, STH administered in three injections (20 μg/injection) and five injections (50 μg/injection) pro-

Table I. *Effects of porcine growth hormone (STH) on total length growth expressed in % of initial total length increment in* Discoglossus pictus.
Number of animals in brackets

μg of STH per injec.	Number of injec.	Larval stage	Initial length (mm)	% total length increment
5	5	35-38	19-23	25.7±2.6 * (8)
10	5	35-38	19-23	30.9±3.6 * (6)
20	5	35-38	19-23	28.0±3.0 * (10)
Control 1	5	35-38	19-23	29.6±2.6 (8)
50	3	32-35	16-21	19.2±3.0 * (9)
Control 2	3	32-35	16-21	19.7±2.6 (8)

* $P > 0.05$.

Table II. *Effects of porcine growth hormone (STH) on total length growth expressed in % of initial total length increment in* Rana ridibunda. .
Number of animals in brackets

μg of STH per injec.	Number of injec.	Larval stage	Initial length (mm)	% total length increment.
5	3	30	9-11	16.2±1.8 * (7)
Control 1	3	30	9-11	4.6±1.1 (5)
5	3	31	11-15	22.0±2.1 ** (7)
Control 2	3	31	11-15	14.0±2.0 (7)

* $P < 0.01$; ** $P < 0.02$.

Table III. *Effects of porcine growth hormone (STH) on total length growth expressed in % of initial total length increment in* Rana iberica.

Number of animals in brackets

μg of STH per injec.	Number of injec.	Larval stage	Initial length (mm)	% total length increment.
20	3	35-38	30-35	17.3±1.8 * (6)
Control 1	3	35-38	30-35	6.6±1.5 (6)
50	5	38-44	32-35	12.9±1.6 * (9)
Control 2	5	38-44	32-35	4.1±0.8 (7)

* $P < 0.01$.

duces, in respect to the control animals, a significative increment in total length and body length ($P < 0.01$). The correspondent results are shown in Table III (Fig. 3).

Figures 1, 2 and 3 show a strong pigmentation darkening which was appreciated in all cases a few hours after administration of the hormone. This reaction of pigmentation darkening has never before been described in papers on the subject.

In the case of *Rana iberica*, the percentage of animals that performed metamorphosis was bigger in the control animals, at a time of the experiment, than in those treated with STH porcine. We think therefore, that this hormone probably inhibits metamorphosis.

Discussion

The majority of the studies performed on the effects produced by administration of prolactin and mammalian growth hormone (STH) on amphibia made a great number of investigators assume that a hormone similar to mammalian prolactin is the most responsible of growth in amphibia at larval stage.

BROWN and FRYE (5) found that the lowest dosis of STH porcine necessary to obtain a significative length increment in *Rana pipiens* tadpoles was 50 μg/day for a period of two weeks. We have found similar results with porcine growth hormone on *Discoglossus pictus*. These results brought BROWN and FRYE (5) to the conclusion that only prolactin has proved to be effective as stimulant of growth in amphibian tadpoles, and that the positive response to mammalian STH, found by other investigators, may be due to contamination with prolactin. Studies already in mind may help us to find out which are the roles of either hormone in *Discoglossus pictus*.

Nevertheless, ENEMAR *et al.* (9) assure that STH is more effective as growth stimulant in *Rana temporaria* tadpoles than prolactin, and attribute the contradictory results obtained by other authors (2, 3) to having used growth hormone from a different species of mammal. According to these authors the differences in molecular structure between bovine STH and ovine STH (14) may have as important a role as to cause the difference in growth in anuran amphibia tadpoles. Our experiments on *Rana ridibunda* and *Rana iberica* lead us to think that porcine STH administered in as low doses as 5 μg and 20 μg, in two and three injections, has activity on both species before metamorphosis.

Comparing the results obtained with STH porcine on *Discoglossus pictus* tadpoles on one hand and on *Rana ridibunda* and *Rana iberica* on the other, we concluded that the different responses obtained depend on the species of amphibia used for the experiment.

We point out that inhibition of metamorphosis only occurred when STH was administered during the last stages of larval growth.

According to BROWN and FRYE (6), the fact that only STH and not prolactin has effect after metamorphosis, suggests that the primary mechanism of growth control

may be different in these two stages of the life cycle. This different response must reflect a basic difference in the mechanism of tissue response to the hormone and it could be due to either of the two following factors: 1) The same tissues respond to prolactin and STH, but change their relative sensibilities to both hormones during metamorphosis or 2) Different tissues or cellular groups show response to the two hormones and there is a change in the proportions or quantities of tissues specially sensible to STH and prolactin during metamorphosis. Bioassays of adult anuran amphibia have shown the presence of prolactin-like (7, 8, 12, 16, 18) and STH-like (22) activities. BROWN and FRYE (5) think that the pituitary gland of amphibian tadpoles produces at least one necessary factor for normal growth and based on the results obtained with exogeneous prolactin, support ETKIN and GONA (10) and BERN *et al.* (3) on the idea that normal growth and development during the stages prior to metamorphosis is regulated by a balance between a prolactin-like hormone and a thyroid hormone which stimulate larval growth on one hand and metamorphosis on the other.

We think that regulation of growth in amphibian tadpoles depends, on the activity of a somatotrophic hormone (STH-like, prolactin-like) on one hand and a thyroid hormone on the other. It is probable that after metamorphosis there may be a change in the sensibility of the tissues to the somatotrophic hormones, or that the activity of these hormones is inverted.

We point out, on the action of STH porcine on growth of *Rana iberica* tadpoles that total number of proteins per animal increases in the animals treated with STH, whereas DNA does not seem to perform a significative increment. The above results support the idea that cells increase in size but not in number.

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