

EFFECTS OF PROLONGED ADMINISTRATION OF LARGE DOSES OF ACTH ON BUFO MELANOSTICTUS TADPOLES

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Background and Methods: The present study reports the effects of different ACTH preparations, in similar and different concentrations on Bufo Melanostictus Tadpoles. **Results:** ACTH administration proved antagonistic to tadpole growth hormone. There seemed to be no dose-effect response when ACTH was administered in 0.02 IU/ml instead of 0.01 IU/ml. Similar ACTH concentrations with difference of manufacture showed varied biological potency. **Conclusions:** This perhaps indicates loss of effectiveness due to change from ACTH 1-24 to ACTH 1-18 to ACTH 1-10 in descending order.

Keywords: Tadpole, ACTH, Growth

INTRODUCTION

It is known that antagonism exists between the individual hormones of the anterior lobe. A number of direct observations show that adrenocorticotrophic hormone (ACTH) antagonises growth hormone (STH), thyroid stimulating hormone (TSH) and gonadotropic hormone.¹ One of the causes for this state of mutual opposition stems from the increased synthesis of ACTH. This indicates that excess ACTH is produced at the expense of other pituitary hormones. As a consequence of this enhanced discharge of ACTH, secretion of STH is inhibited.² However, it has been reported that intravenous administration of synthetic or porcine (but no bovine) ACTH leads to a transient elevation of plasma growth hormone concentration in adults.^{3,4} Therefore, the purpose of the present study presents the effects of prolonged administration of large doses of ACTH on Bufo Melanostictus tadpoles.

MATERIAL AND METHODS

Field ponds usually contain tadpoles of several simultaneous hatchings; collection of such animals indicate variability in the standards of experimental parameters. In order to avoid such variations, numerous Bufo melanostictus tadpoles belonging to single hatchings were collected separately, from a variety of ponds at the University Campus. After bringing them to the laboratory, the tadpoles were divided into suitable groups and were given refined suspension of parboiled spinach.⁵ Thus they were allowed to acclimatise for a day or so. Premetamorphic tadpoles were used in these experiments, since physiological connections between pituitary and hypothalamus are established by the time they reach stage 52 of the normal table of *Xenopus laevis*.⁶⁻⁸ The tadpoles were selected with the help of a binocular microscope. After measurement, individuals of the same size were randomly divided into comparable groups of control and test.

Growth Correlations

It may be stated that during development, there appears to be a gradual unfolding with time of anterior pituitary and receptor-gland hormones; that regulate the final size, form and body parts. This elaboration of growth is indicative of a second phase of specific constitutional growth potential provided by the genotype of the egg. During this phase, the growth regulating hormones gradually take over the function of coordination and regulation of final size.⁹ However, growth is also dependent upon environmental factors; since nutrients, oxygen supply,¹⁰ and temperature have an effect on the physico-chemical background¹¹ in which the synthetic processes of growth take place. Therefore, growth of Bufo melanostictus tadpole can be worked out by measuring its body size. As matter of fact, a decisive evidence of growth has also been sought recently through the use of these measurements.¹²

Method of Measurement

Since, growth follows the expression of size and weight, the growth of Bufo melanostictus tadpole can be worked out by measuring its body size. The method adopted for this purpose was similar to that described by Ahmad and Mukarram.¹²⁻¹⁴ In order to measure each tadpole, a nylon net was used to introduce a tadpole into a 6 cm diameter glass Petri dish in the absence of water. As soon as the tadpole ceased its wriggling movements; the Petri dish was tilted slightly towards the head side. By sliding, the tadpole body was shaped into a straight line. The Petri dish was placed on a glass bearing a millimetre grid and the length of the tadpole, from head to tail was noted.

Design of Experiments

Space is a major factor in the growth rate of tadpoles and crowding has been reported to accelerate metamorphosis by operating through the hypothalamic mechanism in anuran tadpoles.¹⁴⁻¹⁵ therefore, animals thus measured were divided at random into 6 groups of 20 individuals each and kept

in separate tanks.¹⁶ Thus, test and control groups were maintained in similarly healthy conditions.

It is known that growth ceases from the direct inhibition of pituitary functions following the cachexia of inanition and under feeding. Thus for feeding, 60 grams of parboiled spinach was liquefied with a small quantity of dechlorinated water and the volume was made up to 1000 ml by the addition of water. Each day, 1 ml of this suspension per tadpole was introduced into each tank.^{5,16,18}

Since, temperature is a measure of molecular agitation, it limits the rate of chemical reactions and thus it is one factor in the control of growth and metabolism.² Tadpoles correspond in body temperature to that of their environment. Any fluctuation in the environmental temperature will have a direct bearing on the chemical reactions in the larval body. Enzymatic reactions are greatly influenced even by alteration of the aquatic temperature.¹⁹

Small aquatic animals, especially if their muscular activity is low, have a body temperature that closely approximates to that of the surrounding water. As might be expected, the temperature of a small plastic tank exposed to environmental temperature from all directions, compared to that of a pond, is easily alterable. Thus the amount of water is an important agent in the acquisition of surrounding temperature. Thus, a rise or fall of temperature causes oscillations in the metabolic activity of the tadpole.^{2,15}

In brief it was, therefore, in the interest of the investigation to maintain the external temperature at which the species normally lives and that in which the *Bufo melanostictus* tadpoles have lived recently. Since, higher temperatures favour thyroid stimulating hormone (TSH) over the growth factor production in pituitary and accelerate metamorphosis.²⁰⁻²¹ Therefore, the tadpoles were kept under fairly constant temperature (28±1° C) during the experimental period.²²

RESULTS

An examination of the data of experiment 1 (table, 1) indicates that *Bufo melanostictus* tadpoles maintained for 11 days in a solution of 0.01 IU/ml ACTH showed retarded growth. The effect of the dose was visible from the 2nd day of the treatment. The difference of growth between control and test 1 became more dominant with the advance of time. By the 11th day test 1 tadpoles were 0.57 mm shorter than control, although the difference of mean lengths between test and control groups was non significant. On the other hand, test 2 tadpoles maintained in a solution of 0.02 IU/ml ACTH showed slower growth than test 1 tadpoles. The effect of ACTH was more obvious from the next day of treatment, however

tadpoles showed a non significant reduction of mean body length in comparison to control group. Thus test 2 tadpoles were 0.49 mm shorter than control but 0.08 mm larger than test 1 tadpoles.

A consideration of the data of experiment II (table 2) indicates that test 1 tadpoles maintained for ten days in 0.02 IU/ml ACTH showed a non significant regressed growth, whereas, test 2 tadpoles maintained in a similar solution of a different preparation showed a significant retarding effect than test 1 tadpoles (p<0.05, t-test). Thus, test 2 tadpoles were 4.11 mm and 3.74 mm shorter than control and test 1 respectively.

Hind limbs were present in 65% control of experiment I by day 9; in 70% by day 10 and 100% by day 11. Controls also showed 10% erupted fore limbs by day 11. Hind limbs were present in 5% test 1 tadpoles by day 9; in 65% by day 9 and 70% by day 11. While, hind limbs were present in 20% of test II tadpoles by day 9 and in 70% by day 10. However, 10% showed emerged for limbs.

Tadpoles of both the tests showed more or less the same size.. All the test animals showed similar retardation of head, eyes trunk and tail.

Despite the ACTH solutions being made from the same stock; the affect of higher doses, failed to produce a difference in size between the tests. Moreover, there was non-significant difference between the control and tests. However, test tadpoles were somewhat darker than controls as the most significant extra-adrenal action of ACTH is the stimulation of release of melanocyte stimulating hormone.²³⁻²⁴

Hind limbs were present in 10% controls of experiment I by day 8; in 65% by day 9 and emergence of fore limbs in 10% by day 10. On the other hand, 50% test 1 showed hind limbs by day 9 and 60% by day 10. However hind limbs were absent in test II.

Table-1: Effect of 0.01 and 0.02 IU/ml *ACTH on the growth of premetamorphic *Bufo melanostictus* tadpoles. (Body length (mm) as mean± SD, n=20)

Days after treatment	Control Normal	Test-I 0.01 IU/ml	Test-II 0.02 IU/ml
1	9.00±0.0	9.00±0.0	9.00±0.0
2	10.00±0.0	9.95±0.22	10.00±0.0
3	11.60±0.50	11.40±0.60	11.85±0.37
4	13.30±0.47	13.25±0.55	13.30±0.47
5	14.75±0.44	14.50±0.61	14.75±0.44
6	16.50±0.61	16.40±0.60	16.47±0.41
7	18.20±0.62	17.95±0.90	17.95±0.60
8	19.40±0.75	18.85±1.04	18.95±0.89
9	20.05±0.89	19.05±0.94	19.20±1.15
10	20.10±0.79	19.40±0.94	19.40±0.94
11	20.17±0.92	19.60±0.94	19.68±1.00

*ACTH supplied by Armour Company.

The difference in mean length of control, as well as both the tests, was statistically significant. Some of the tadpoles of test I remained shorter than others, indicating more sensitivity to the hormone. On the other hand, tadpoles of test II remained much smaller than control and showed a pale pigmentation.

Table-2: Effect of 0.02 IU/ml ACTH on the growth of premetamorphic *Bufo melanostictus* tadpoles. (Body length (mm) as mean± SD, n=20)

Days after treatment	Control Normal	Test-I 0.01 IU/ml	Test-II 0.02 IU/ml
1	6.00±0.0	6.00±0.0	6.00±0.0
2	7.10±0.31	6.90±0.45	6.50±0.51
3	8.25±0.55	8.15±0.67	7.05±0.40
4	9.75±0.85	9.60±1.10	7.63±0.68
5	11.10±1.16	11.00±1.30	8.42±0.69
6	12.45±1.54	12.45±1.50	8.94±1.06
7	13.90±1.89	13.75±2.00	9.11±1.68
8	15.55±2.19	14.90±2.24	10.28±1.54
9	16.21±2.02	15.75±2.38	12.33±1.78
10	17.11±1.91	16.74±2.60	13.00±1.31

*ACTH prepared by SIGMA, ** ACTH supplied by FREDERIKSBERG CHEMICAL LAB. LTD. COPENHAGEN

DISCUSSION

Presently, the most important use of ACTH is, as a diagnostic agent in adrenal insufficiency. For this purpose ACTH is administered and if there is no acute response; prolonged administration can be carried out.²⁵⁻²⁶ ACTH used for experiment I was less potent and is available as a sterile solution, but the other two preparation obtained in the form of lyophilized powder were more potent than the first one. Test 2 tadpoles of experiment 2 showed hypersensitive reactions to the last preparation.

Investigations on the structural activity relationships of ACTH indicate that the 24 amino-acid fragment of ACTH; i.e. ACTH 1–24 is equipotent or slightly more potent than the parent 39 amino-acid molecule.²⁷⁻²⁸ The C-terminal sequence, starting with amino acid 25 is a vestigial appendage and has no biological role. The peptide from ACTH 1–24 to ACTH 1–18 is associated with a decrease in biological potency.²⁸⁻²⁹ By removal of one or more of the dibasic amino acids at position 15 to 18 a great reduction in potency follows.³⁰⁻³² ACTH 1–10 in large doses stimulates steroidogenesis in hypophysectomised rats³¹ and is possibly the “active center” of the molecule. Nevertheless the grouping of the dibasic amino acids is one of the important affinity sites of the ACTH molecule. Thus, the three ACTH preparations studied in this investigation are possible indicative of the potencies of the ACTH fragments, ACTH 1–10, ACTH 1–18 and ACTH 1–24 respectively.

It is known that the overproduction of ACTH^{4,33} stimulates the normal adrenal glands to produce

excessive amounts of glucocorticoid. ACTH administered exogenously the resulting secretions of glucocorticoids and aldosterone³⁰ were also involved in suppressing the growth hormone action in test tadpoles.

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