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[COMMUNICATION]

Dependence of Prolactin-Stimulated Tail Fin Growth and Molting on Water in Male Salamanders (*Hynobius nigrescens*)

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ABSTRACT—Prolactin (PRL)-stimulated tail fin growth and molting are dependent on water in male *Hynobius nigrescens.* Tail length and height increased in PRL-injected groups regardless of habitat (i.e., terrestrial or aquatic), but the aquatic habitat helped the rapid growth of tail fin. Even under low-temperature conditions (mimic breeding season), molting was speeded up in all saline-injected animals housed in a fully aquatic habitat, and further speeded up by PRL regardless of habitat, suggesting that an aquatic environment induced the secretion of PRL in this species.

INTRODUCTION

It is known that in *Triturus cristatus carnifex* [1], *Notophthalmus viridescens* [2], and *Cynops pyrrhogaster* [3] prolactin (PRL) increases tail height; but we do not know whether PRL stimulates an increase in tail length, perhaps due to difficulty in comparing tail lengths. We [4] observed that in *Hynobius lichenatus* many individuals possessed variously regenerated tails, suggesting that the tail breakage occurs in other tail-unautotomizable species: tail length may be valid when making a comparison of its change in the same individual. So we added in this study tail length data to PRL-stimulated parameters using animals housed individually. Also, in winter-dormant male *H. nigrescens* kept in a terrestrial habitat, tail height does not show any noticeable increase following the administration of 5 IU PRL [5]. This first suggests that the absorption of water from an aquatic environment is necessary for the growth of tail fin. To examine this possibility, we conducted an experiment in a fully aquatic or terrestrial habitat.

Molting in amphibians usually occurs every few days and is speeded up at higher temperatures [6]. It is known that in urodeles molting is induced by thyroid hormone and/or PRL [1, 7]. However, a difference in molting according to habitat (i.e., terrestrial or aquatic) is unknown. We also report here water-dependent (without PRL) or habitat-independent (with PRL) molting in *H. nigrescens* under low-temperature conditions.

MATERIALS AND METHODS

Adult male *H. nigrescens* were collected from a pond in Iwamuro-mura, Niigata Prefecture, Japan (37°44′N, 138°50′E; 180 m elevation) during the breeding season of March 1989. After emergence from the water they were raised in large, shaded terraria located outdoors at Niigata University and provided with terrestrial isopods and earthworms as food.

On 18 July, the animals maintained were transferred from the terraria to a room regulated at 8°C, and here experiments were conducted from 21 July to 8 August. They were not fed during this period. A temperature of 8°C was settled as a mimicry of the breeding season. The males used were seasonally immature [8]. They were divided into four groups (n=7/group): (1) aquatic-saline (AS), (2)

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aquatic-PRL (AP), (3) terrestrial-saline (TS), and (4) terrestrial-PRL (TP). In groups AS and AP, the animals were enclosed individually in a plastic box (21 cm long, 14 cm wide, and 3.5 cm deep with a lid) nearly filled with aged tap water; and in groups TS and TP, in a box with a moist paper towel. The animals were injected intraperitoneally with a saline solution (0.6% NaCl aq.) in groups AS and TS, or 5 IU ovine PRL dissolved in the saline solution in groups AP and TP every other day. The PRL (Sigma Chemical Co., St. Louis) used had a mean potency of 31 IU/mg. The volume of fluid injected was always 0.1 ml. Prior to the injection, the animals were anesthetized with 0.1% MS 222 solution, weighed to the nearest 0.1 g with a Roberval's balance, and measured to the nearest 0.1 mm with slide calipers. The following data were recorded: snout-vent length, measured from the tip of the snout to the posterior angle of the vent; tail length, from the posterior angle of the vent to the tip of the tail; head width, at the broadest part; and tail height, at the highest part. The experimental animals were brought back to their own box immediately after the injection, and awoke from the anesthesia in approximately 0.5–2 hr. The occurrence of molting was monitored at the time of injection.

If the data taken in a parameter showed a meaningful change (i.e., increase or decrease), the paired *t*-test was used for a comparison between initial and final values in that parameter (alpha= 0.05).

RESULTS AND DISCUSSION

Snout-vent length, body mass, and head width did not show a meaningful change in any group. A rapid gain in body mass occurs due to water uptake and head width increases greatly after entering a breeding pond in male *H. nigrescens* [9]. We were unable to induce these phenomena in seasonally "immature" males with the administration of PRL. However, PRL stimulates an increase in head width in some of seasonally "mature" male *H. nigrescens* [5].

In groups AS, AP, and TP, tail length and height increased significantly, but not in group TS (Table 1). Their increase in group AS was very small. In all in group AP and some in group TP, the tail faintly assumed a fin-like appearance. The basal and dorsal part of the tail stood out a little in these animals. In group AP, tail length and height increased linearly and extremely until the tenth day after the beginning of injection, and thereafter plateaued (Fig. 1). In group TP, they increased slowly from the sixth day until the end of the experiment. The rate of increase in tail height in group AP was nearly twice as great as that in group TP at the end of the experiment. Increased and plateaued tail height in group AP was not so great as that seen in typical aquatic phase males during the breeding season, and the rate of increase in tail height in group AP was similar to that at the transition between females before and after entering the water [9]. Kikuyama *et al.* [3] stated concerning *C. pyrrhogaster* that the simultaneous administration of estradiol blocked the PRL-induced increase in tail height. That is, PRL

TABLE 1. Changes in tail length and height (mean ± SE) in groups aquatic-saline (AS), aquatic-prolactin (AP), terrestrial-saline (TS), and terrestrial-prolactin (TP) at 8°C, as shown by initial and final (in parentheses) values

Group	n	Tail length (mm)	*P*	Tail height (mm)	*P*
AS	7	55.1±1.0 (57.5±1.1)	0.001	7.9±0.2 (8.1±0.2)	0.02
AP	7	54.7±2.2 (60.4±2.6)	0.001	8.0±0.2 (9.0±0.2)	0.001
TS	7	61.4±2.0 (61.5±2.0)	NS	8.1±0.1 (8.0±0.1)	NS
TP	7	51.4±2.8 (55.2±3.1)	0.001	8.2±0.2 (8.7±0.2)	0.01

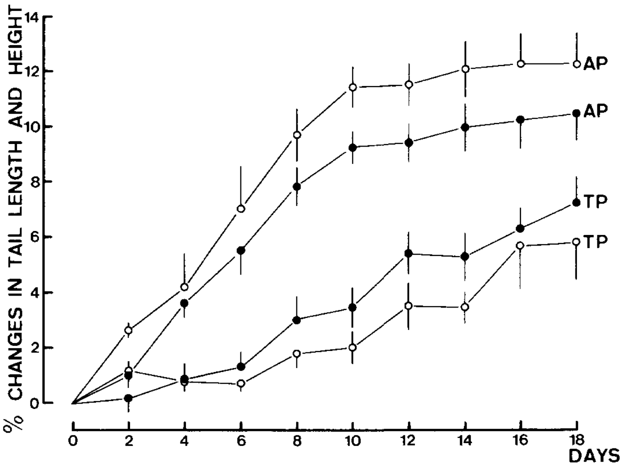

FIG. 1. Prolactin-stimulated increase in tail length (solid circle) and height (open circle) in groups AP and TP (see the caption of Table 1) at 8°C. Vertical bars denote SE on either side of the mean.

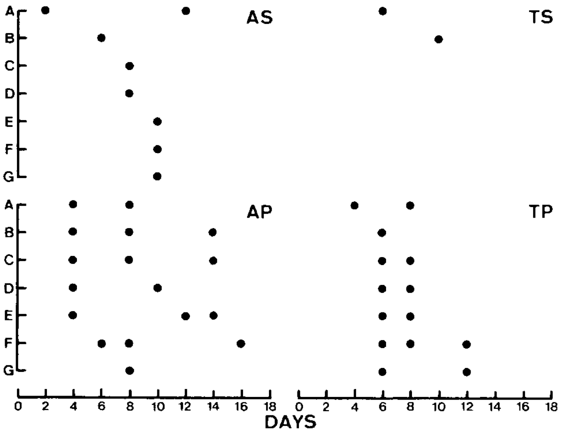

FIG. 2. The frequency of molting (solid circle) in groups AS, AP, TS, and TP (see the caption of Table 1) at 8°C. A-G: individual numbers.

increases tail height in the male, but not in the female because of the higher concentration of estradiol. In *H. nigrescens*, however, plasma concentrations of estradiol are at the base in females both before and after entering a breeding pond (Hasumi, Iwasawa, and Nagahama; unpublished data) when tail height increases only a little [9]. These lowest concentrations of estradiol therefore suggest that androgen is involved in the greater growth of male tail fin (sexual dimorphism) of this hynobiid species, although PRL alone increases tail height in several salamandrids [1–3]. An extremely high concentration of testosterone is determined in male *H. nigrescens* in the process of tail fin growth (Hasumi, Iwasawa, and Nagahama; unpublished data). An aquatic habitat appears unnecessary for the PRL-stimulated growth of tail fin, but assists its rapid growth. If a high level of PRL is seen in animals during the terrestrial phase, a little growth of tail fin must occur at that time independently of entering the water. Accordingly, the absence of PRL-stimulated increase in tail height of winter-dormant male *H. nigrescens* kept in a terrestrial habitat [5] may be due to the comparison of a little increase in tail height of these animals with no increase in that of control ones; the comparison between tail heights of the same individual was made in the present study.

Regardless of the type of treatment (i.e., saline or PRL), all animals housed in a fully aquatic habitat molted at least once during the experimental period (Fig. 2). Regardless of habitat (i.e., terrestrial or aquatic), PRL induced frequent molting. Smooth molting occurred in group AP, but not in group TP. Vellano *et al.* [1] reported that in thyroidectomized *T. cristatus* PRL induced molting. Dent *et al.* [7] stated for *N. viridescens* that PRL alone did not induce molting but might facilitate molting when introduced with thyroid hormone. Probably PRL, apart from thyroxine, stimulates molting in *H. nigrescens*. Because the present July materials were seasonally immature [8], concentrations of the hormones associated with aquatic reproduction (e.g., PRL [10]) would be expected to be low. Despite this expectation, even under low-temperature conditions, molting was speeded up in all saline-injected males housed in a fully aquatic habitat in comparison with those in a terrestrial one. On the other hand, most of those saline-injected, mature male *H. nigrescens* that temporarily submerge in water possess the surface layer (cornified epidermis), indicating that temporary submergence is not enough to induce molting [5]. All of these observations lead us to postulate that a fully aquatic environment induces the secretion of PRL in this species.

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