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Changes in Plasma and Pituitary Prolactin Levels in Toad (*Bufo japonicus*) Larvae during Metamorphosis

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ABSTRACT—Both plasma and pituitary prolactin (PRL) concentrations in *Bufo japonicus* tadpoles at various developmental stages were determined by a homologous radioimmunoassay. Plasma PRL levels continued to rise gradually as metamorphosis proceeded, the values at late climax being about 3 times higher than those at the premetamorphic stage. PRL concentrations and the amount of PRL in the pituitary gland also increased during the preclimax period and reached a maximum at mid-climax with a slight decline at the end of metamorphosis. PRL synthesis, as measured by incorporation of [^{3}H]leucine into pituitary PRL *in vitro*, was relatively low during premetamorphosis, continued to rise throughout the prometamorphic period, reached a maximum at climax and declined at the end of metamorphosis. Taken together, these data indicate that the function of the pituitary gland in toad larvae in terms of PRL secretion is gradually enhanced as metamorphosis progresses. The present results obtained with toad tadpoles and those obtained previously with *Rana catesbeiana* larvae are discussed, taking the differences in metamorphic pattern between the two species into consideration.

INTRODUCTION

The discovery that mammalian prolactins (PRLs) exert anti-metamorphic [1] and growth-promoting [2, 3] effects on amphibian larvae prompted a hypothesis that PRL levels are high during premetamorphosis and prometamorphosis and begin to decline once metamorphosis has started, so that tadpoles grow during the preclimax period and metamorphose rapidly once the animals have reached climax [4]. However, with regard to bullfrog (*Rana catesbeiana*) larvae, it has been found that plasma PRL values obtained by homologous radioimmunoassay do not fit this hypothesis. Immunoassayable PRL levels are relatively low during the preclimax period [5] and begin to rise at mid-climax when the tissues have undergone considerable transformation through the effect of thyroid hormone [5, 6]. Up to now, plasma PRL levels in anuran larvae have been determined only for *R. catesbeiana*. Recently, a homologous radioimmunoassay for toad (*Bufo japonicus*) PRL has been developed [7]. It is therefore of interest to see whether the plasma PRL levels in toad tadpoles, which are comparatively small and undergo complete metamorphosis rapidly, exhibit changes similar to those in bullfrog larvae, which metamorphose rather slowly with a long growth phase. Changes in the PRL contents and PRL synthesis in the pituitary gland during metamorphosis were also studied.

MATERIALS AND METHODS

Animals

Eggs of *B. japonicus* collected in the suburbs of Tokyo were hatched in our laboratory at 23°C. Tadpoles were fed on boiled spinach. The stages of metamorphosis were classified according to Limbaugh and Volpe [8].

Plasma and pituitary samples

Blood was taken from tadpoles by insertion of a heparinized capillary into the heart. Each sample (about 200–400 μl) was collected from 20–30

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animals at the same developmental stage. After collection, blood samples were centrifuged and the plasma was stored at −70°C until use. The anterior pituitary gland was quickly dissected out under a dissecting microscope. Each sample consisting of 10 pituitaries taken from animals at the same developmental stage was homogenized with a Teflon homogenizer in 500 μl distilled water and stored at −70°C until assay.

PRL radioimmunoassay

PRL for antiserum production, radioiodination and use as a reference standard was purified from anterior pituitary glands of adult toads [9]. Antiserum against the toad PRL was raised in a female rabbit by the multiple-site injection technique [10]. Radioiodination of toad PRL with $Na^{125}I$ (carrier-free; The Radiochemical Centre, Amersham, England) was carried out at room temperature according to the modified lactoperoxidase method [11]. The specific radioactivity of the radioiodinated PRL was about 40–50 μCi/μg. A 30% specific binding of the added radioligand was obtained in the absence of any unlabeled PRL when the antiserum was used at a final dilution of 1:20,000. Details of the radioimmunoassay have been described elsewhere [7]. It had been confirmed that the neurointermediate lobe homogenate and mammalian PRL and growth hormone did not cross-react with the antiserum, and that the plasma of hypophysectomized toads showed the least degree of cross-reaction. Sensitivity of the radioimmunoassay averaged 0.12 ng per 100 μl of assay buffer. The interassay coefficient of variation was 9.2% and the intraassay coefficient of variation 8.0%.

Determination of PRL synthesis in the pituitary gland

PRL synthesis in the pituitary glands from tadpoles at various developmental stages was measured *in vitro*. Whole pituitary glands from 50 tadpoles at the same developmental stage were put into a vial containing 150 μl of 67% Eagle's MEM in which the leucine concentration was reduced to 10% and supplemented with 1 μCi [^{3}H]leucine (NEN; spec. act., 145 Ci/mmol). The pituitary glands were incubated at 25°C in a Dubnoff-metabolic shaking incubator gassed with 95% O_2-5% CO_2. After incubation, the glands together with the medium were homogenized. An aliquot of the homogenate was used for protein determination. To the homogenate, an equal volume of 5% polyacrylamide sample gel (pH 8.6) was added. The mixture was homogenized and layered on the top of polyacrylamide gels. Electrophoresis was performed according to the procedure described elsewhere [12]. After electrophoresis, the gel was stained with amido black 10B in 7% acetic acid for identification of the PRL band. The band was dissected out, placed in a screw-capped vial containing 200 μl H_2O_2, and heated in an oven at 80°C. After solubilization, scintillation fluid (Aquasol 2, NEN) was added to the vial and radioactivity was measured in a liquid scintillation counter.

Statistical analysis

Statistical analysis was performed using Student's t-test.

RESULTS

As indicated in Figure 1, plasma PRL levels rose gradually as metamorphosis progressed. The average value at the end of metamorphosis (stage 46) was about 3 times higher than that at the premeta-

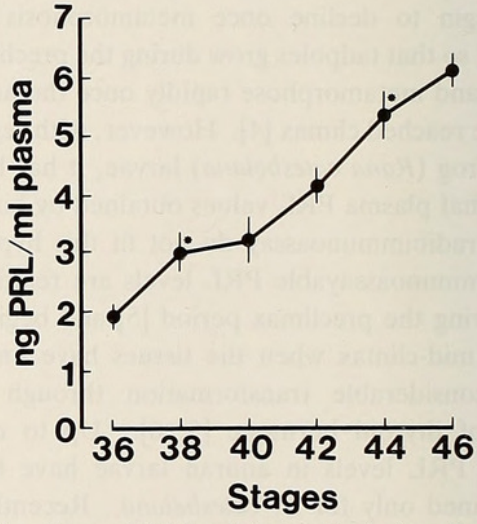

FIG. 1. Plasma PRL levels in toad tadpoles. Each point and vertical line represent the mean of 15 determinations and standard error of the mean, respectively. * Significantly different from preceding value at 5% level.

morphic stage (stage 36).

Both the total amount and concentration of PRL in the pituitary gland were low at stage 36, but increased throughout the prometamorphic stages and reached a maximum at mid-climax (stage 44). After completion of metamorphosis, the concentration but not total amount of pituitary PRL declined significantly (Fig. 2).

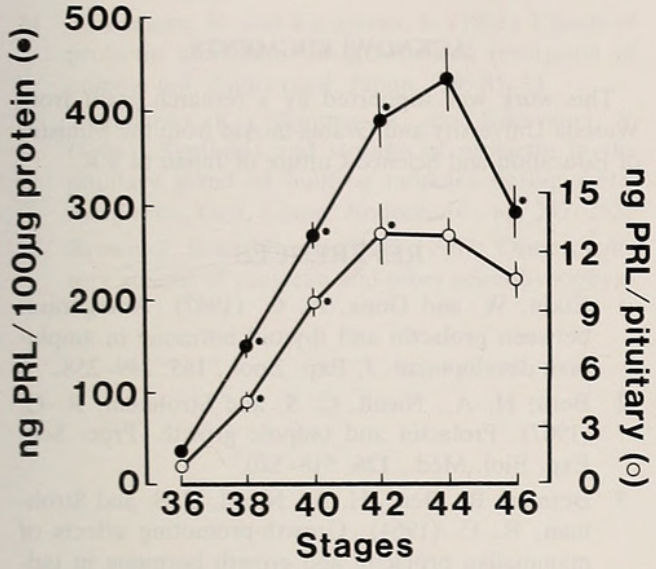

FIG. 2. Changes in PRL levels in the anterior pituitary of toad tadpoles at various developmental stages. Each point and vertical line represent the mean of 13 determinations and standard error of the mean, respectively. * Significantly different from preceding value at 5% level.

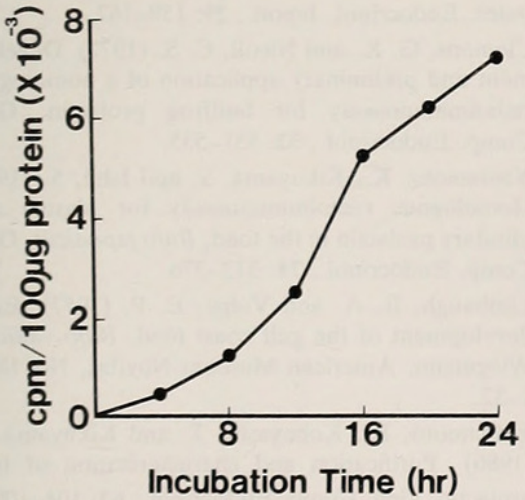

FIG. 3. Time course of incorporation of [^{3}H]leucine into prolactin in the pituitary glands of toad larvae. Whole pituitary glands were incubated with [^{3}H]leucine for 24 hr at 25°C. PRL in the medium and pituitary glands was separated by disc gel electrophoresis, and the radioactivity of the PRL band was measured. Each point represents the mean of 2 determinations.

Pituitary glands of stage 46 animals were incubated in the presence of [^{3}H]leucine. The incorporation of the isotope into PRL increased over a period of 24 hr (Fig. 3). PRL synthesis in the pituitary glands of tadpoles at various stages was measured by monitoring the incorporation of [^{3}H]leucine into PRL for 20 hr. PRL synthesis was found to be enhanced during prometamorphosis and remained high during mid-climax. At the end of metamorphosis, PRL synthesis declined considerably (Fig. 4).

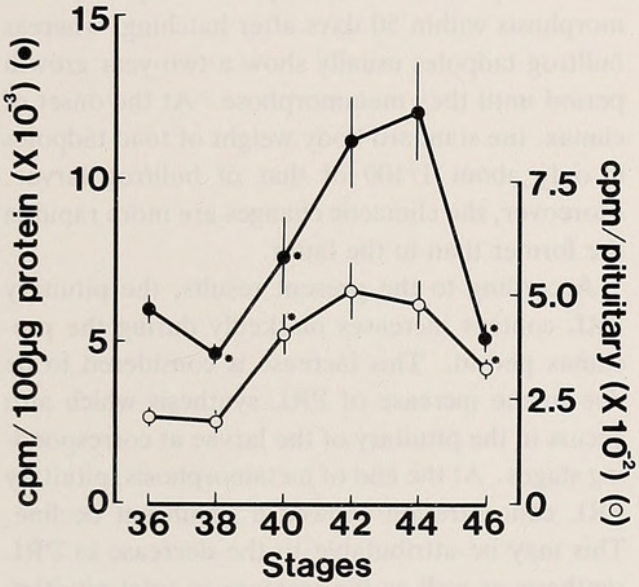

FIG. 4. PRL synthesis in the pituitary glands of toad tadpoles during metamorphosis. Whole pituitary glands from tadpoles at various developmental stages were incubated with [^{3}H]leucine for 20 hr at 25°C. Radioactivity of electrophoretically separated PRL from the medium and pituitary glands represents PRL synthesized in the pituitary glands during the incubation period. Each point and vertical line represent the mean of 6 determinations and standard error of the mean, respectively. * Significantly different from preceding value at 5% level.

DISCUSSION

The present experiment revealed that plasma PRL levels in toad tadpoles are initially low, and then rise as metamorphosis proceeds. In the case of bullfrog tadpoles, the plasma PRL level is low and remains rather constant until the animals

reach the climax stage [5]. During the early climax stages, elevation of the PRL level is not so conspicuous. Then the level rises markedly during mid-climax and reaches a maximum at late climax [5, 6]. Although the PRL levels in toad tadpoles continue to rise throughout the larval period, they are about 1/10–1/20 of the levels in bullfrog tadpoles [5] at comparable stages. Endogenous PRL is known to have antimetamorphic and growth-promoting activities [5, 13, 14]. Accordingly, the difference in PRL levels between the two species may reflect the difference in the larval size as well as the duration of the larval period between the two species. Toad tadpoles complete metamorphosis within 50 days after hatching, whereas bullfrog tadpoles usually show a two-year growth period until they metamorphose. At the onset of climax, the standard body weight of toad tadpoles is only about 1/100 of that of bullfrog larvae. Moreover, the climactic changes are more rapid in the former than in the latter.

According to the present results, the pituitary PRL content increases markedly during the pre-climax period. This increase is considered to be due to the increase of PRL synthesis which also occurs in the pituitary of the larvae at corresponding stages. At the end of metamorphosis, pituitary PRL concentration showed a significant decline. This may be attributable to the decrease in PRL synthesis as well as the increase in total pituitary protein.

As in the case of the plasma PRL level, the pituitary PRL concentration is less than 1/10 of the value in bullfrog larvae [15]. It was also revealed that the pituitary PRL concentration in toad larvae is much lower than in the adult. On the other hand, the maximum plasma concentration in the larvae is comparable to the concentration in non-breeding (terrestrial) adult toads, which is lower than that in breeding (aquatic) toads [7]. PRL is known to be involved in osmoregulation in amphibians [16–18]. It is often observed that toad tadpoles at late climax stages, which have undergone a considerable transformation for terrestrial life, become edematous and can not survive when kept in water, while bullfrog tadpoles at corresponding stages can stay in an aquatic environment. This may come from the difference in the plasma PRL levels between the two species at late climax.

In conclusion, PRL synthesis and release in toad tadpoles are enhanced as metamorphosis progresses. However, their pituitary function in terms of PRL secretion seems to be lower than that of bullfrog tadpoles or adult toads.

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REFERENCES

1 Etkin, W. and Gona, A. G. (1967) Antagonism between prolactin and thyroid hormone in amphibian development. J. Exp. Zool., **165**: 249–258.

2 Bern, H. A., Nicoll, C. S. and Strohman, R. C. (1967) Prolactin and tadpole growth. Proc. Soc. Exp. Biol. Med., **126**: 518–520.

3 Berman, R., Bern, H. A., Nicoll, C. S. and Strohman, R. C. (1964) Growth-promoting effects of mammalian prolactin and growth hormone in tadpoles of *Rana catesbeiana*. J. Exp. Zool., **156**: 353–360.

4 Etkin, W. (1970) The endocrine mechanism of amphibian metamorphosis, an evolutionary achievement. Mem. Soc. Endocrinol., **18**: 137–155.

5 Yamamoto, K. and Kikuyama, S. (1982) Radioimmunoassay of prolactin in plasma of bullfrog tadpoles. Endocrinol. Japon., **29**: 159–167.

6 Clemons, G. K. and Nicoll, C. S. (1977) Development and preliminary application of a homologous radioimmunoassay for bullfrog prolactin. Gen. Comp. Endocrinol., **32**: 531–535.

7 Yamamoto, K., Kikuyama, S. and Ishii, S. (1989) Homologous radioimmunoassay for plasma and pituitary prolactin in the toad, *Bufo japonicus*. Gen. Comp. Endocrinol., **74**: 373–376.

8 Limbaugh, B. A. and Volpe, E. P. (1957) Early development of the gulf coast toad, *Bufo valliceps* Wiegmann. American Museum Novitas, No. 1842, 1–32.

9 Yamamoto, K., Kobayashi, T. and Kikuyama, S. (1986) Purification and characterization of toad prolactin. Gen. Comp. Endocrinol., **63**: 104–109.

10 Vaitukaitis, J., Robbins, J. B., Nieschlag, E. and Ross, T. (1971) A method for producing specific antisera with small doses of immunogen. J. Clin. Endocrinol., **33**: 988–991.

11 Sakai, S., Kohmoto, K. and Johke, T. (1975) A receptor site for prolactin in lactating mouse mammary tissues. Endocrinol. Japon., **22**: 379–387.

12 Kikuyama, S., Yamamoto, K. and Mayumi, M. (1980) Growth-promoting and antimetamorphic hormone in pituitary glands of bullfrogs. Gen. Comp. Endocrinol., **41**: 212–216.

13 Clemons, G. K. and Nicoll, C. S. (1977) Effects of antisera to bullfrog prolactin and growth hormone on metamorphosis of *Rana catesbeiana* tadpoles. Gen. Comp. Endocrinol., **31**: 495–497.

14 Yamamoto, K. and Kikuyama, S. (1982) Effects of prolactin antiserum on growth and resorption of tadpole tail. Endocrinol. Japon., **29**: 81–85.

15 Yamamoto, K., Niinuma, K. and Kikuyama, S. (1986) Synthesis and storage of prolactin in the pituitary gland of bullfrog tadpoles during metamorphosis. Gen. Comp. Endocrinol., **62**: 247–253.

16 Brown, P. S. and Brown, S. C. (1987) Osmoregulatory actions of prolactin and other adenohypophysical hormones. In "Vertebrate Endocrinology: Fundamentals and Biomedical Implications Vol. 2". Ed. by P. K. T. Pang and M. P. Schreibman, Academic Press, New York, pp. 45–84.

17 Hirano, T., Ogasawara, T., Bolton, J. P. Collie, N. L., Hasegawa, S. and Iwata, M. (1987) Osmoregulatory role of prolactin in lower vertebrates. In "Comparative Physiology of Environmental Adaptation". Ed. by R. Kirsch and B. Lahlou, Karger, Basel, pp. 112–124.

18 Olivereau, M., Olivereau, J. M., Kikuyama, S. and Yamamoto, K. (1990) Hypothalamo-hypophysial axis in osmoregulation. In "Fortschritte der Zoologie Vol. 38. Biology and Physiology of Amphibians". Ed. by W. Hanke, Gustav Fischer Verlag, Stuttgart, pp. 371–383.

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