Effect of Prolactin Antiserum on Growth and Resorption of Tadpole Tail

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Abstract

Rabbit antiserum to bullfrog prolactin (A/S) or normal rabbit serum (NRS) was administered to premetamorphic bullfrog larvae. Collagen synthesis in the tail fin was not affected by A/S. However, the enhancement of collagen synthesis induced by pimozide treatment was completely blocked by A/S. Premetamorphic tadpoles kept in $3 \times 10^{-8} \text{M}$ thyroxine received A/S or NRS. A/S accelerated tail resorption. The results indicate that the endogenous prolactin is acting as an antimetamorphic hormone, although it is not certain that normally circulating level of prolactin contributes to promoting the growth of the tail fin in larval bullfrogs.

Administration of mammalian prolactin to tadpoles accelerates growth (Berman et al., 1964) and inhibits metamorphosis (Etkin and Gona, 1967). The tadpole tail is a typical target organ of prolactin. Prolactin increases the tail size (Berman et al., 1964). Associated with this, the hormone stimulates the development of connective tissue in the tail and synthesis of macromolecules such as collagen and acid mucopolysaccharide found in the tissue (Yoshizato and Yasumasu, 1970, 1971). Prolactin also inhibits thyroid hormone-induced tail resorption (Etkin and Gona, 1967; Derby and Etkin, 1968).

Pituitary glands of both adult and larval bullfrogs are known to contain a factor(s) which stimulates the development of connective tissue and the synthesis of collagen in the tail fin (Yoshizato et al., 1969, 1972) and blocks the tail resorption (Hsu et al., 1976; Yamamoto et al., 1979). It has been postulated that the growth-promoting and antimetamorphic factor(s) is released from the tadpole pituitary gland (Etkin and Lehrer, 1961; Etkin et al., 1969). Recently we demonstrated that a fraction with properties similar to mammalian prolactin, which was electrophoretically separated from bullfrog pituitary glands, has growth-promoting and antimetamorphic activities and concluded that the fraction contains bullfrog prolactin (Kikuyama et al., 1980).

The bullfrog prolactin was purified from adult bullfrog pituitary glands (Yamamoto and Kikuyama, 1981) and the rabbit antiserum to the prolactin (A/S) was prepared in our laboratory. The effect of A/S on the collagen synthesis in the tail fin and thyroxine($T_4$)-induced shrinkage of the tail in larval bullfrogs was examined in order to see whether endogenous prolactin is actually involved in larval growth and metamorphosis.

Materials and Methods

Prolactin used for antiserum production was purified from adult bullfrog adenohypophyses as described previously (Yamamoto and Kikuyama, 1981). The antisera were made in four white rabbits. Each animal was injected subcutaneously with 1 mg of prolactin.
dissolved in 1.5 ml of saline emulsified in the same volume of Freund's complete adjuvant 3 times at 14-day intervals. Two weeks after the last injection, all animals were bled. It was ascertained that the antisera thus obtained did not show a precipitin line in agar diffusion with several mammalian prolactins and growth hormones and a growth hormone fraction of adult bullfrog pituitary origin but did show such a line with the prolactin prepared from adult bullfrog pituitary glands (Yamamoto and Kikuyama, 1982).

Homogenate of 10 pituitary glands from stage X tadpoles was subjected to disc gel electrophoresis. After electrophoresis, the gel was placed on a plate which was stacked with agar. In parallel to the disc gel, a groove, 1 mm in width, was cut. Into the groove 100 µl antiserum was placed in order to examine its cross-reactivity with the larval bullfrog prolactin.

Premetamorphic bullfrog tadpoles (stage X) were injected with 0.05 ml of A/S or normal rabbit serum (NRS) every other day, 3 times altogether. Another group of tadpoles received 3 injections of 25 µg pimozide dissolved in 0.05 ml of saline containing 0.3% tartaric acid in addition to A/S or NRS. Collagen synthesis in the tail fin was measured according to the method of Yoshizato and Yasumasu (1970). Following the day of the last injection, the tail fin was dissected out and incubated with 2 ml of medium containing 0.2 µCi of 14C-proline (specific activity: 295 mCi/mmol) for 3 hr. 14C-Labeled collagen was extracted by the method of Fitch et al. (1955) and the radioactivity was measured in a scintillation counter.

Premetamorphic bullfrog tadpoles were kept in 3 x 10^-8 M T4 and injected with 0.1 ml of A/S or NRS every 5 days. Another group of tadpoles were kept in tap water and received similar injections of NRS. Tail length and maximum tail height were measured on day 0 and from day 12 till day 25 at intervals of 1-4 days. The degree of tail length or tail height change was expressed as the percent change from the original value.

Results

As shown in Fig. 1, a single precipitin line appeared between A/S and a protein band which is known to possess a potent prolactin activity (Kikuyama et al., 1980).

The A/S did not affect collagen synthesis in premetamorphic tadpoles. Incorporation of 14C-proline into collagen fraction of the tail fin of A/S-injected animals did not differ significantly from that of NRS-injected ones. In both groups, there were no notable changes in tail height and tail length between the value at the beginning of treatment and that at sacrifice. In the animals treated with pimozide and NRS, collagen synthesis was enhanced remarkably. The enhancement of collagen synthesis was completely blocked when NRS was replaced with A/S (Fig. 2). In the pimozide plus NRS injected group, tail height increased by 5.5% of the original value but tail length remained almost unchanged. In the tadpoles injected with pimozide and A/S, both tail length and tail height were not changed throughout the experiment.

In the tadpoles kept in T4 and treated with A/S, both tail length and tail height decreased rapidly as compared with the tadpole, kept in T4 and injected with NRS (Fig. 3A B and 4). In these two groups, the front limbs emerged almost simultaneously. In the A/S-treated group and NRS-treated group front limbs came out 15.3 ± 0.6 (mean ± SE) and
Fig. 2. Effect of A/S on collagen synthesis in the tail fin of normal and pimozide-treated premetamorphic tadpoles. Animals were injected with 0.05 ml NRS, NRS plus 25 μg pimozide (Pim), 0.05 ml of A/S or A/S plus Pim 3 times every other day. Collagen synthesis was determined according to the method of Yoshizato and Yasumasu (1970). Each bar represents mean±SE for 6 animals. The value of NRS plus Pim was significantly different from the value for other groups at 5% level (Student’s t-test).

15.1±0.5 days after the animals were put into T4 solution, respectively.

In control animals kept in tap water and injected with NRS, no metamorphic changes occurred (Fig. 4). The tail remained almost unchanged (Fig. 3A, B).

Discussion

The present results of agar diffusion test together with the result of radioimmunoassay employing A/S in which purified adult bullfrog prolactin and pituitary homogenates or plasma samples from larval bullfrog gave the same pattern of inhibition curve (Yamamoto...
Tadpoles treated with A/S and kept in T\textsubscript{4} exhibited precocious tail resorption compared to the animals treated with NRS and kept in T\textsubscript{4}. Controls which were injected with NRS and kept in tap water are still in the premetamorphic stage. 

and Kikuyama, 1982), indicate that tadpole prolactin possesses immunological properties similar to those of adult prolactin.

When prolactin is administered to premetamorphic tadpoles every other day, the collagen synthesis in the tail fin reaches a plateau 5 days after the first injection (Yoshizato and Yasumasu, 1970). At this time, a slight enlargement of the tail is already recognizable. In the present experiments, the effects of A/S and/or pimozide on collagen synthesis were examined similarly. A/S did not lower the collagen synthesis in premetamorphic tadpoles. On the other hand, the enhancement of the collagen synthesis by pimozide, a dopamine blocking agent which is known to stimulate the release of prolactin from the bullfrog pituitary gland (Seki and Kikuyama, 1982) was suppressed by A/S. Therefore, it is evident that the injected A/S was effective in neutralizing the bioactivity of the endogenous prolactin released tonically. It is probable that the A/S-induced decline of bioactivity of chronically released prolactin is not reflected in the collagen synthesis until a little longer time than 5 days. According to our data obtained recently (Yamamoto and Kikuyama, 1982), the plasma prolactin level measured by radioimmunoassay is relatively low in the premetamorphic period. Therefore, the possibility will not be denied that the degree of attenuation of bioactivity of the endogenous prolactin by A/S is too low to be detected by measuring the collagen synthesis in premetamorphic tadpoles.

The effect of A/S on T\textsubscript{4}-induced tail resorption was evident. In this case, the effect of prolactin is cumulative. Accordingly, the A/S-induced decline in the bioactivity of endogenous prolactin is expected to become apparent as metamorphosis progresses. At an advanced metamorphic stage, i.e., the early climax stage, the immunoreactive prolactin level reaches double that at the premetamorphic stage (Yamamoto and Kikuyama, 1982). If the prolactin level in the tadpoles undergoing metamorphosis in the T\textsubscript{4} solution is elevated as in the spontaneously metamorphosing specimens, A/S will reduce the bioactivity of prolactin considerably, judging from the results that A/S nullified the pimozide-induced elevation of bioactivity of prolactin, and thus induces a precocious tail resorption.

Clemons and Nicoll (1977) have reported that spontaneous metamorphosis of bullfrog tadpoles was also accelerated by a rabbit antiserum to the electrophoretically separated bullfrog prolactin. Kikuyama and Seki (1980) demonstrated that dopamine agonists such as ergocornine and bromocryptine, which suppress prolactin release, accelerated T\textsubscript{4}-induced metamorphic changes in bullfrog tadpoles. Therefore, it is evident that prolactin is being released to slow the metamorphic changes during the larval period. Further studies will be needed to clarify to what extent the endogenous prolactin is making a contribution to the growth of tadpoles.
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References


