proceeds slowly, and in no instance did the butterflies collected in these samples disintegrate on handling.
16. The areas of each colony were computed by azimuths and boundary distances with the use of a double meridian distance program from Hewlett-Packard's HP-25 Applications Program (1975).
17. On the basis of repeated observations at all sites, discussions with local residents, and the fact that site Beta was fully formed on 26 November 1977, we estimate 1 December as the date of colony stabilization. Shifting occurs prior to this and some consolidation occurs afterward, but the latter appears minor. Moreover, site fidelity, as in California (2), appears great: the 1977–1978 center of Alpha$_1$ differed by less than 50 m from where it was in 1976–1977.
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Prolactin Receptors in *Rana catesbeiana* During Development and Metamorphosis

Abstract. *Specific binding of ovine prolactin was found in microsomal preparations of tail, gill, and kidney of the bullfrog* Rana catesbeiana. *Binding by larval and adult liver and by kidney before larval stage XVII was low or nondetectable. Renal binding increased during metamorphic climax and in response to treatment with thyroid hormone. The emergence of renal binding of prolactin may signify a shift in the hormone's participation in the control of hydromineral homeostasis from the gill, which is resorbed, to the kidney. A renal action of prolactin during climax may facilitate metamorphosis.*

In the vertebrates, two of the basic functions of prolactin are control of growth and development and the regulation of water and electrolyte balance (*1*). Among the Amphibia, the hormone participates in the control of growth and retention of larval structures (*2*). In this regard, prolactin opposes the metamorphosis-inducing actions of thyroid hormones in larval and neotenic amphibians. In euryhaline teleosts prolactin acts at several sites to maintain hydromineral integrity; it seems to be particularly important in preventing tissue hydration in freshwater habitats (*3*). If one considers the similarities in osmotic stress experienced by freshwater teleosts and aquatic amphibians (especially larval forms), it is not surprising that there is some evidence for a role of prolactin in salt and water homeostasis in amphibians (*4*). The transformation of the bullfrog (*Rana catesbeiana*) tadpole from an aquatic to an amphibious creature must occur in a manner that is compatible with the changing demands on its osmoregulatory capabilities. In view of the involvement of prolactin in growth, development, and osmoregulation, one might expect the hormone to play an important role in the process of metamorphosis as well as in the regulation of growth of larval amphibians. Indeed, some investigators have speculated that developmental patterns may result, in part, from prolactin-controlled shifts in hydromineral homeostasis (*1*, *5*). However, the extent of this control by prolactin has not been fully ascertained (*4*, *6*). Furthermore, the amphibian organs whose activity might be influenced by prolactin have not been identified.

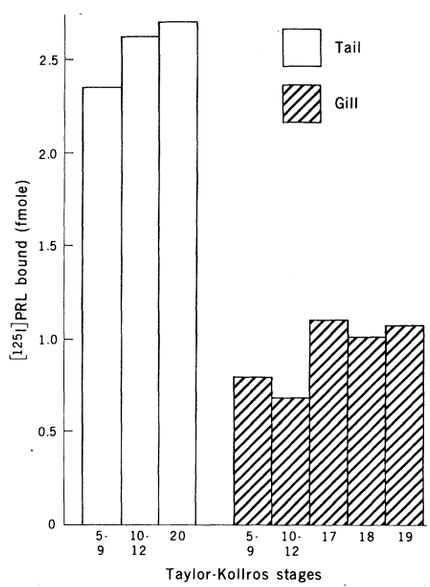

Fig. 1. Saturable binding of ^{125}I-labeled ovine prolactin (*PRL*) by tail and gill microsomal fractions. Values are femtomoles of hormone bound by 0.5 mg of membrane protein in the presence of 90 fmole of label. Arabic numbers are used instead of the more conventional Roman numerals for the different stages.

Studies of the binding of labeled prolactin to presumptive receptors in membrane fractions of mammalian (*7*) and avian (*8*) tissues indicate that the hormone shows a high degree of specific binding to established and suspected target organs. The degree of binding generally varies directly with the functional status of these organs. Hence, analysis of the occurrence of prolactin receptors in amphibian tissues should provide information on probable target organs of the hormone. However, the only information on this subject is a brief report that membranes from adult bullfrog kidney show a high degree of specific binding of ^{125}I-labeled ovine prolactin (*9*). We have studied target organ responsiveness to prolactin during amphibian development and metamorphosis. The potential sensitivity of organs in larval and adult bullfrogs to prolactin was evaluated by using the relative amount of saturable binding of ^{125}I-labeled ovine prolactin to microsomal fractions as an index of organ sensitivity. The structures examined were the tail and gill, both of which occur only in the larva, and the liver and kidney, which persist in the adult. The gill and kidney are potential sites for hydromineral-regulatory actions of prolactin, and the tail is a known target organ of the hormone.

Tadpoles of *R. catesbeiana* (11 to 20 g) were kept in tap water at 18°C with a constant food supply. After 1 week, they were staged according to Taylor and Kollros (*10*) and killed, the four structures being removed and placed in Dry Ice. The organs of two groups of 30 tadpoles each (stages V to IX and X to XII) were pooled by organ type and stage range. The organs of 15 to 20 tadpoles were pooled by type for each stage between XVII and XX and for stage XXII. The kidneys and livers of 12 adult bullfrogs were pooled by organ type. Membrane fractions of the kidney pools were prepared twice for stages XX and XXII and three times for stages V to IX and adult.

Microsomal fractions of each tissue pool homogenate were prepared by differential centrifugation according to the method of Shiu *et al.* (*11*). The initial low-speed (600*g*) centrifugation was omitted in order to minimize tissue loss. The 100,000*g* pellet was resuspended in 25 m*M* tris and 15 m*M* $CaCl_2$ buffer, *p*H 7.6. Membrane protein was estimated according to the Hartree modification of the Lowry method (*12*).

Ovine prolactin (*13*) was labeled with ^{125}I by a lactoperoxidase procedure (*14*). Labeled hormone was separated from labeled enzyme and free ^{125}I by column

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chromatography with Sephadex G-100 Superfine gel (1 by 55 cm) equilibrated in assay buffer at 4°C. The specific activity of the label was estimated from the percentage transfer and ranged from 65 to 85 μCi/μg. The presence of saturable binding in microsomal fractions was tested by incubating the fractions with increasing amounts of labeled hormone in the presence or absence of 1.0 μg of unlabeled prolactin. The assay conditions were those of Shiu *et al.* (*11*) with a 16-hour incubation period at room temperature.

Displaceable binding of prolactin was present in tail, gill, and some kidney microsomal preparations (Figs. 1 and 2), and dose-related competitive inhibition of binding of a fixed amount of labeled prolactin was observed in the presence of increasing amounts of unlabeled hormone (data not shown). No binding was detected in liver membranes from either tadpoles or adults. The extent of binding by the tail was consistently greater than that by the gill, and it remained constant throughout the larval period in both organs.

In contrast to the two exclusively larval structures, prolactin binding by kidney fractions from premetamorphic and early prometamorphic tadpoles is very low or absent (Fig. 2). Consistently detectable binding appeared in the kidney in late prometamorphosis and increased throughout metamorphic climax. In agreement with the earlier study (*9*), binding was high in adult kidney preparations. The binding appears to be relatively specific for prolactin in that electrophoretically separated bullfrog prolactin, but not purified bullfrog growth hormone, can displace the ovine prolactin label (*15*). The use of prolactins and growth hormones from several mammalian species also indicates specificity of these sites for lactogenic hormones (data not shown).

The renal binding of prolactin increased at a time when circulating thyroid hormone concentrations were also rising (*16*). Accordingly, we examined the possibility of a causal relationship between these phenomena by measuring the extent of prolactin binding by tail, gill, and kidney after the animals were exposed to thyroxine. Two groups of 20 tadpoles each (stages X to XII) were kept in 24-liter aquariums at 22°C with a constant food supply. They were given no treatment or were exposed to thyroxine (50 ng per milliliter of aquarium water) for 24 hours. One week after thyroxine treatment, the tadpoles were killed and the three tissues pooled by type from the two groups for membrane preparation and assay. This experiment was repeated with the tadpoles being killed 2 weeks after exposure to thyroxine. Under these conditions, the control tadpoles showed no developmental changes whereas the experimental tadpoles advanced two to three stages. In addition, two groups of six adult bullfrogs were injected intraperitoneally with triiodothyronine (T_3; 10 ng per 0.1 ml of 0.01*N* NaOH) or solvent every other day for 6 days (three injections total) and the kidneys were pooled by group, processed, and assayed for binding.

Exposure to thyroxine greatly increased renal prolactin binding in the tadpole (Fig. 3). Thus, the increased concentrations of circulating thyroid hormones during metamorphic climax probably induce, directly or indirectly, the appearance of prolactin receptors in the

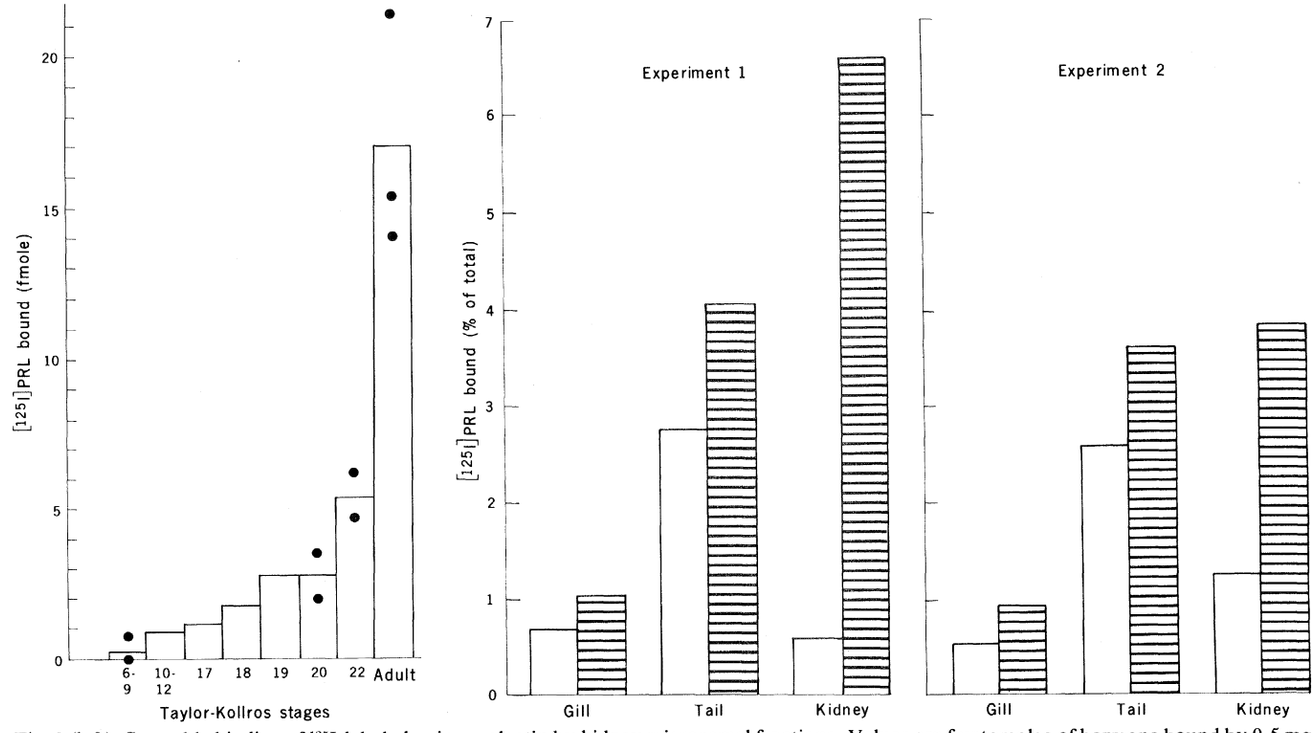

Fig. 2 (left). Saturable binding of ^{125}I-labeled ovine prolactin by kidney microsomal fractions. Values are femtomoles of hormone bound by 0.5 mg of membrane protein in the presence of 90 fmole of the label. The tops of the vertical bars represent the value of a single determination or the mean of two or three measurements. When more than one estimate was obtained, the individual values are presented as closed circles. Arabic numbers are used for the different stages. Fig. 3 (right). Effects of thyroxine exposure on saturable binding of ^{125}I-labeled prolactin by gill, tail, and kidney microsomal fractions. Binding was assayed in the presence of 300 fmole of labeled hormone, and values were adjusted to 1 mg of membrane protein. Blank bars represent data from control groups and striped bars represent those from thyroxine-treated groups. The height of each bar was determined by subtracting the mean of four binding values obtained in the presence of 1 μg of unlabeled prolactin (unsaturable or nonspecific binding) from the mean of four binding values obtained in the absence of unlabeled hormone (total binding). Intraassay precision was such that only differences between control and experimental groups of tail ($P < .01$) and kidney ($P < .01$) fractions were significant. The animals were killed either 1 week (experiment 1) or 2 weeks (experiment 2) after they were exposed to thyroxine.

kidney. The administration of T_3 also increased renal binding by about 20 percent in the adult bullfrog (data not shown). A similar thyroid hormone effect has been found in the kidney of adult rats (*17*). The small increase in prolactin binding by the tail and the lack of a significant effect on the branchial receptors may be due to the concomitant regressive changes that thyroid hormone causes in these structures.

The following findings indicate that the binding of prolactin by the renal, branchial, and caudal membrane preparations is of biological importance in *R. catesbeiana*. (i) The presence of binding in the tail is consistent with studies indicating a growth-promoting or antimetamorphic action of the hormone on this structure during premetamorphosis and prometamorphosis (*2*). (ii) The binding sites are saturable and, at least with respect to the adult kidney, display specificity and high affinity (*18*) for prolactin. (iii) The inability to detect displaceable binding in most liver preparations is consonant with the absence of effects of the hormone on hepatic tissue hydration (*5*) and the activity of certain hepatic enzymes, and the failure of ovine prolactin to block thyroid hormone induction of these enzymes (*19*). Although these observations do not prove that the hormone has no influence over any hepatic function in this species, a tentative conclusion can be made that the binding sites exhibit tissue specificity.

The presence of displaceable binding in gill tissue and kidney leads one to speculate on a possible contribution of the hormone to the control of hydromineral homeostasis in this species. In some freshwater teleosts prolactin decreases sodium efflux and water permeability in the gill, increases urine flow, and decreases urine sodium (*3*). Administration of the hormone corrected the hyponatremia in hypophysectomized *Necturus maculosus* and *Ambystoma mexicanum* and antagonized thyroxine-induced lowering of plasma sodium concentration in *Notophthalmus viridescens* (*4*). In *R. catesbeiana* tadpoles, the concentration of circulating prolactin and the metamorphic rate are altered by environmental salinity (*20*). Also in tadpoles of this species, injection of prolactin antiserum lowered the plasma sodium concentration (*21*).

It has been suggested that the antimetamorphic effects of prolactin may be mediated, in part, through its control of hydromineral balance. A recent study of neotenic *Ambystoma tigrinum* indicated that the larval tissue dehydration may initiate transformation into the adult form (*5*).

In this context, it is interesting that prolactin binding increases in the kidney at the time when the rate of larval structure regression is greatest. Since prolactin may have a role in tadpole osmoregulation, it is reasonable to assume that it may increase urine production (that is, water elimination), or sodium retention, or both, through these newly required renal receptors. These changes in renal function, in turn, may alter plasma and tissue electrolyte concentrations sufficiently to facilitate thyroid hormone effects on cellular activities involved in the final stages of metamorphosis. In fact, plasma osmolarity increases at the time of metamorphic climax in this species (*22*).

Thyroid hormones themselves are responsible for the transepidermal (mucosal to serosal) active transport of sodium that appears during metamorphic climax in bullfrog tadpoles (*23*).

Most investigators have not considered the possibility of a metamorphosis-favoring role of prolactin. Some data indicate that circulating levels and pituitary concentration of prolactin increase during late climax in *R. catesbeiana* (*24*). Furthermore, prolactin receptors appear in the kidney in response to thyroid hormones (Fig. 3), which are potent inducers of metamorphosis. Also, in the majority of previous studies of prolactin's role in metamorphosis, tadpoles were treated with the hormone during premetamorphosis and prometamorphosis, times that are characterized more by growth than by transformation. During these stages, prolactin binding exists in the tails and gills, but is very low or absent in the kidney. Prolactin is synergistic with thyroid hormones in metamorphosis of other amphibians (*25*). Thus, prolactin may favor retention and growth of larval structures while exerting a branchial osmoregulatory influence during most of the tadpole period. With metamorphosis, the hormone's role as a growth-promoting agent is lost (*26*) along with its function at the gill level. The coincident emergence of renal receptors indicates that the osmoregulatory function may persist beyond the larval period, shifting from the gill to the kidney where the hormone may act to facilitate the metamorphic process.

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