Fish and Amphibian Models for Developmental Endocrinology

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ABSTRACT

The hormonal control of ontogeny in fish and amphibians is reviewed. Neuroendocrine regulation and actions of metabolic and osmoregulatory hormones (thyroid, interrenal, pituitary, pancreas, and gut) during amphibian and fish metamorphosis and the parr-smolt transformation of juvenile salmon are considered. The developmental significance of hormones in egg yolk is discussed. It is concluded that the transitions between life-history stages provide many unique opportunities for studying basic endocrine phenomena.

The study of hormones in ontogeny is particularly interesting and challenging because the endocrinology of the animals under investigation is continually changing through time, e.g., the embryo progresses to larva, juvenile, and adult. The transitions between life-history stages provide many unique opportunities for studying basic endocrine phenomena. For example, during development a tissue can gain or lose status as an endocrine target, and such events can be exploited to investigate mechanisms and control of cellular responses to hormones. The endocrine system not only influences and directs development, but also is itself a subject of a developmental program. Complex neuroendocrine systems evolve from simpler ones, and this can also present valuable opportunities for research. Studies in developmental endocrinology can focus on the appearance of hormones in endocrine tissue, the appearance and disappearance of hormone receptors in endocrine targets, changes in the functions of hormones during development, the ontogeny of hormone feedback mechanisms, and other regulatory processes. In contrast, endocrine studies of adult animals are usually conducted with the assumption that the basic function and control of the endocrine glands and their relationships with the target tissues are unchanging.

As alternates to mammalian models, there are numerous examples in fish and amphibian ontogeny that are appealing to the endocrinologist. The best known and most utilized of these are metamorphoses. Fish and amphibian metamorphoses include many dramatic and quantifiable developmental changes that are under hormone control. The metamorphosis of the tadpole into the adult frog, and its control by thyroid hormones, have been a long-standing subject of investigation. It was perhaps the first "model" used to study hormonal control of ontogeny (Guderianatsch, '12). Generations of developmental endocrinologists have been trained with this model and its investigation continues to yield important insights into the actions, interactions, and mechanisms of action of hormones in development. Several parallels to amphibian metamorphosis and its hormonal control occur in fish, including flounder metamorphosis and the parr-smolt transformation of migratory salmonids. The more recent investigations of fish and amphibians emphasize the multihormonal nature of developmental control mechanisms.

With respect to the earliest stages of development, fish and amphibians have been less well studied. Thyroid hormones have long been known to accelerate early development of fishes and, with the discovery of significant amounts of thyroid hormones in fish egg yolk and embryos, the role of hormones in fish embryogenesis has become an active area of research. Information on the role of hormones in amphibian embryogenesis is more limited. This is unfortunate, since we
have known for over 20 years that amphibian embryos can respond to hormonal treatments with some of the biochemical changes underlying metamorphosis (Tata, '68).

On a morphological basis, some early embryonic stages of mammals, birds, reptiles, and amphibians are similar in appearance. This evolutionary conservatism of early development may derive from the fact that mutations which alter early development are likely to have profound deleterious effects (through ontogenetic amplification) on anatomical features of later developmental stages (reviewed by Gould, '83). If this argument holds for hormonal control of early development, there should be extensive commonality in endocrine mechanisms among vertebrates. Thus, there may be a firm foundation for extrapolation of results from models of endocrine control in fish and amphibian development to that of higher vertebrates. Accordingly, the developmental endocrinology of fish and amphibians is relevant to mammalian endocrinologists engaged in biomedical and veterinary research or in studies related to animal husbandry. Of course, investigation of these "more primitive" forms has obvious heuristic interest from the viewpoint of comparative endocrinology, i.e., the study of evolutionary patterns of hormonal control mechanisms.

Collectively, fish and amphibians comprise one-half of the living vertebrates and they exhibit tremendous diversity with respect to developmental patterns. These include, as examples, direct development and metamorphosis, as well as fetal-maternal relationships which even involve a placenta in some forms.

Studies of the hormonal control of fish and amphibian development should provide useful models and information for a variety of reasons: 1) they are alternatives to mammalian models; 2) comparisons of embryonic and post-embryonic transformations may reveal common developmental mechanisms and thereby advance our basic understanding of developmental processes; 3) studies of free-living embryos facilitate the dissection of fetal-maternal relationships; and 4) on a practical level, development in an aqueous milieu makes them easy to culture and manipulate experimentally. Finally, in some cases (e.g., amphibian metamorphosis) researchers can draw upon a detailed base of knowledge regarding their developmental endocrinology.

Fish ontogeny has been modeled as a hierarchical system of intervals consisting of periods or phases and steps (Balon, '84). According to this saltatory model of ontogeny, the developmental periods of fish or amphibians can be divided into embryonic, larval, juvenile, and adult stages. Periods are separated by thresholds which represent times of highly accelerated rates of development. The endocrine control of morphogenesis and physiology during developmental periods and thresholds provides informative models for understanding the endocrinology of vertebrate development. The most detailed studies available have concentrated on amphibian metamorphosis and the parr-smolt transformation of juvenile salmonids.

**METAMORPHOSIS**

Endocrine control of the transformation of larval to juvenile amphibians has been classically recognized as being under the antagonistic control of pituitary prolactin (PRL) and thyroid hormones (White and Nicoll, '81). Larval growth and development are promoted by PRL, whereas metamorphosis is triggered by thyroid hormones with probable facilitation by glucocorticoids. PRL opposes thyroid hormone action (anti-metamorphic activity) in tadpoles. Here we summarize selected recent developments in the endocrinology of metamorphosis.

It has long been known that administration of thyroid hormones can trigger metamorphosis or metamorphic changes in vivo. The concept that they do so in nature is supported by the demonstration of a surge in thyroid hormones in blood during metamorphic climax in both anurans and urodèles (Miyauchi et al., '77; Regard et al., '78; Mondou and Kaltenbach, '79; Suzuki and Suzuki, '81; Larras-Regard et al., '81; Weil, '86; Albrech et al., '86; Norman et al., '87). It has been assumed that the increase in thyroid hormones during metamorphosis is due to increased stimulation of the thyroid by pituitary thyroid-stimulating hormone (TSH). An increase in the volume of immunohistochemically identified TSH cells has been observed near metamorphic climax of *Bufo calamita* and *Rana perezi* (Garcia-Navarro et al., '88). Although it is apparent that the thyroid is more active near metamorphic climax, increases in circulating levels of TSH have not yet been demonstrated. Part of the mechanism for increasing thyroid hormone levels during metamorphosis may be an increase in the capacity of the thyroid for hormone production in response to TSH. Increased response capacity of the thyroid during or after metamorphosis has been shown for *Am-
bystoma mexicanum (Darras and Kuhn, ’84) and A. tigrinum (Norman and Norris, ’87).

Thyroid hormones may generally act to control synthesis of specific proteins via their interaction with receptors in the cell nucleus (reviewed by Oppenheimer, ’79, ’85). They appear to act similarly in amphibian metamorphosis. In concept, the hormone-receptor interaction is followed by increased transcriptional activity, formation of new messenger RNAs, synthesis of proteins, and cellular responses to the hormone. Putative nuclear receptors for thyroid hormones have recently been described for several tadpole tissues that are responsive to thyroid hormones and their levels have been reported to increase in some tissues during both spontaneous and thyroid hormone-induced metamorphosis (Yoshizato and Frieden, ’75; Galton, ’83, ’84; Moriya et al., ’84; Galton and St. Germain, ’85).

Administration of glucocorticoids accelerates thyroid hormone-induced metamorphosis, and blood levels of corticosteroids are elevated during metamorphosis (Jaffe, ’81; Krug et al., ’83; Jolivet-Jaudet and Leloup-Hatey, ’84; Kikuyama et al., ’86; Carr and Norris, ’88). In vitro exposure of toad and bullfrog tails to aldosterone was reported to increase both fin resorption and nuclear (receptor) binding of thyroid hormone (Kikuyama et al., ’85). These findings suggest that at least part of the metamorphic action of corticosteroids involves direct action to increase target tissue sensitivity to thyroid hormones.

Based on the tadpole growth-promoting and anti-metamorphic activity of PRL, and according to an early model of endocrine control of amphibian metamorphosis (Etkin, ’70), it would be expected that blood levels of PRL would be elevated in amphibian larvae, followed by a decrease during metamorphosis. However, measurements of circulating levels of PRL using homologous assays have revealed low levels during pre- and pro-metamorphosis and a clear elevation in PRL during metamorphosis (Clemens and Nicoll, ’77; Yamamoto and Kikuyama, ’82). Changes in blood levels of PRL, thyroid, and corticosteroid hormones during metamorphosis show slightly different patterns in different amphibian species or in the same species studied in different laboratories. However, a general pattern of hormone change might be illustrated by collation data on the bullfrog from various laboratories (Fig. 1).

The change in blood concentrations of PRL during metamorphosis can be viewed in light of possible PRL functions in larval and adult amphibians (White and Nicoll, ’79). The growth-promoting effects of PRL in tadpoles may be mediated, at least partly, through hepatic production of synlactin (Delidow et al., ’88). The adult frog liver does not appear to produce synlactin (Delidow et al., ’86). Measurement of specific binding of PRL to amphibian tissues has suggested that some larval tissues (gill, tail) have apparent receptors for PRL, as would be expected for hormone-responsive tissues. The amphibian kidney shows low specific binding for PRL in tadpoles, but shows a marked increase in binding capacity in the adult or after thyroid hormone treatment of tadpoles (White and Nicoll, ’79; Tarpey and Nicoll, ’87). It has been suggested by White and Nicoll (’79) that the function of PRL switches from growth-

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Fig. 1. Changes in blood plasma levels of thyroxine (Mondu and Kaltenbach, ’79), corticosterone (Jaffe, ’81), and prolactin (Yamamoto and Kikuyama, ’82) during metamorphosis of the bullfrog, Rana catesbeiana. Developmental stages are indicated according to Taylor and Kolfrs (’46).
promoting in tadpoles to osmoregulatory in adults. Studies of hormone receptors during development illustrate the point that circulating levels of hormones may tell only part of the story; the sensitivity or capacity of tissues to respond to hormones is perhaps more important.

Changes in endocrine tissues of the gut and pancreas during amphibian metamorphosis have been reported (Kaung, '83; L'Hermite et al., '88). Pancreatic 3-cells increase in volume during larval development, decrease during metamorphosis, and then increase again in adult Rana catesbeiana (Farrar and Hulsebus, '88). Tissue-extractable insulin immunoreactivity shows a pattern similar to that of 13-cell volume, and serum insulin-like immunoreactivity peaks before metamorphic climax (Hulsebus and Farrar, '85). Intestinal cholecystokinin (CCK) also increases during larval development, decreases during metamorphosis, and then increases in adult Xenopus laevis (Scalise and Vigna, '88). Changes in insulin during metamorphosis may reflect changes in feeding activity. However, the changes in CCK precede the periods of reduced food intake, which indicates that CCK production may be part of the developmental program. It has been suggested from these studies that CCK may play a role in growth and differentiation of the developing gut and pancreas.

Comparison of endocrine control of metamorphosis between amphibians and fish has been limited to thyroid hormones. Thyroid hormones are reported to induce metamorphosis in the conger eel (Kitajima et al., '67) and flounder (Inui and Miwa, '85). Treatment with the goitrogen, thiourea, blocks flounder metamorphosis, resulting in giant pelagic larvae. Tissue levels of thyroid hormones increase during natural metamorphosis of flounder larvae (Miwa et al., '88). Interestingly, the flounder thyroid appears to have an increased capacity to respond to TSH injections during metamorphosis, similar to what has been shown for two species of Ambystoma during metamorphosis (Inui and Miwa, personal communication). In contrast to results in teleosts, metamorphosis of larval lamprey does not appear to be inducible by thyroid hormone treatment. Circulating levels of thyroid hormones are elevated in larval lamprey, and they decrease during metamorphosis (Wright and Youson, '77). Curiously, induction of metamorphosis has been reported to occur after treating larval lamprey with goitrogens (Suzuki, '87).

**PARR-SMOLT TRANSFORMATION**

The study of juvenile development in fishes offers the opportunity for comparison with the endocrine control of development of earlier stages. In this regard, much information is accumulating on the endocrine changes during the parr to smolt transformation (smolting) of juvenile salmonids. Smolting is the developmental process in which stream-resident juvenile salmonids become adapted to migrate downstream and take up residence in the ocean. Changes in circulating hormone levels during smolting can be summarized (Fig. 2). As in amphibian metamorphosis, plasma hormone levels during smolting may
show variable patterns in different species or in the same species studied under different environmental conditions. The elevation in plasma insulin may function to fortify metabolic reserves that are drawn upon for energy during smolting (Plisetskaya et al., '88). PRL may function as a freshwater osmoregulatory hormone, although its role in smolting deserves more consideration (Richman et al., '87). Thyroxine (T₄) affects many physiological changes associated with smolting. However, treatment of parr with thyroid hormones does not produce all of the changes observed during normal smolting. Thus, thyroid hormone appears to regulate the rate of smolt development, but does not act as a trigger to development of hypoosmoregulatory mechanism (Richman et al., '87). Growth hormone may regulate growth and act in conjunction with cortisol to both mobilize energy reserves and promote development of hypoosmoregulatory mechanism (Richman and Zaugg, '87).

The blood levels of many of the same hormones are seen to change during both salmon smolting and amphibian metamorphosis. It is interesting to speculate to what extent the hormones may have analogous functions in these developmental processes. It is possible that the energetic and metabolic requirements are similar. In both cases energy for tissue differentiation and growth are needed at a time when food availability may be limiting or the animal may not be able to capture or assimilate food. Adjustments in salt and water metabolism may be similar for some species: amphibians changing from aquatic to terrestrial environments or salmon going from fresh- to saltwater. Detailed comparisons of the endocrine developments can be made when more is known about the functions of the hormones during these developmental periods.

The study of hormones in smolting also underscores a fundamental pitfall in endocrine research. While plasma hormone levels clearly change, the interpretation of such data has proved to be somewhat problematic. A peak in a plasma thyroid hormone profile, for example, may be caused by either an increase in secretion or a decrease in clearance from plasma, or both, and plasma clearance is influenced not only by hormone utilization by potentially changing target tissues but also by the excretion of unused hormones. In mammals, although apparently not fishes, multiple pathways of thyroid hormone deiodination may allow for fine-tuning of triiodothyronine (T₃) delivery to sensitive target sites (Eales, '85). Some of the difficulties in correlating generally thyroid-associated events with patterns of plasma thyroid hormones in smolting may be a consequence of potentially important but minimally studied peripheral events. The kinetics of secretion and clearance of T₃ changed markedly throughout the smolting-associated plasma peak (Specker et al., '84). Furthermore, the T₃ content of liver and brain is elevated during times characterized by low plasma T₄ (Brown et al., '89; Specker and Brown, in preparation). These studies suggest that patterns of thyroid hormone utilization may differ from patterns of availability of circulating hormones, and demonstrate that plasma hormone measurements alone can be misleading.

EMBRYONIC DEVELOPMENT

The chemical contributions of oviparous maternal animals to their eggs appear to extend well beyond the simple provision of nutrients. Several hormones have been identified in the yolk of unfertilized fish eggs (Brown and Bern, '89) and these could be involved in the regulation of development. Other potentially important regulatory compounds of maternal origin have been detected in vertebrate eggs, including at least three growth factor messenger RNAs and fibroblast growth factor peptide in Xenopus eggs (see Weeks and Melton, '87; Mercola et al., '88; Kimelman et al., '88). It is particularly relevant to note that maternal platelet-derived growth factor mRNA was detected first in amphibian eggs and later in mouse eggs (Mercola et al., '88). Few if any specific embryonic actions have been established for maternally derived hormones and growth factors that may reach vertebrate eggs and embryos, although a possible role of fibroblast growth factor in mesoderm induction in Xenopus embryos has been proposed (Kimelman et al., '88).

Some recent studies have focused on stimulatory actions of thyroid hormones contained in the yolk of fish eggs (Kobuke et al., '87; Brown et al., '87; Tagawa and Hiran, '87; Greenblatt et al., '89; Brown and Bern, '89), and these suggest that the transfer of hormones from the maternal animal may be vitally important to the offspring (Brown et al., '88). Also, T₃ has been found to be capable of accelerating cell division in a cultured cell line derived from early salmon embryos (Sullivan, unpublished). The chemical profiles of fish eggs include numerous proteins for which no functions have yet been ascribed (Wallace and Selman, '85). It is possible that other bioactive
compounds (e.g., vitamins) may also be incorporated into the eggs of fishes and other vertebrates (Mommsen and Walsh, '87).

The implications of this line of work may be broader than originally thought. If early vertebrate ontogeny is a well-conserved process, and we have every reason to believe that it is, then the specific regulatory actions of maternal chemicals in fish embryogenesis and larval development may be representative of those in early development in utero in mammals. Egg-laying vertebrates provide an entire program of chemical messengers of possible regulatory importance to the egg prior to the time of spawning. In contrast, the mammalian maternal/fetal relationship is potentially more complicated. In addition to placental transfer of hormones, hormones and growth factors. (and/or their mRNAs) could be contributed to the mammalian embryo by oviducal and uterine secretions prior to placentation (see Bern, '90). The fish egg may be valuable as a model for study of the development of mammalian embryos, offering the primary advantages of accessibility and ease of manipulation of the profile of maternally derived compounds to the embryo. The observation that *Xenopus* embryo tissues acquire sensitivity to thyroid hormones long before the onset of embryonic thyroid function (Tata, '68) suggests that amphibian embryos may also be useful in this regard.

In the case of a possible role of maternal thyroid hormones in early mammalian development, it has been difficult to reconcile the contradictory evidence obtained from mammalian studies in utero. Some investigators have concluded that thyroid hormones are of little importance to early fetal thyroid function in utero. Others have suggested that amphibian embryos may also be useful in this regard.

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LITERATURE CITED


