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Corticosteroid signaling in frog metamorphosis

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ABSTRACT

Stress in fetal and larval life can impact later health and fitness in humans and wildlife. Long-term effects of early life stress are mediated by altered stress physiology induced during the process of relaying environmental effects on development. Amphibian metamorphosis has been an important model system to study the role of hormones in development in an environmental context. Thyroid hormone (TH) is necessary and sufficient to initiate the dramatic morphological and physiological changes of metamorphosis, but TH alone is insufficient to complete metamorphosis. Other hormones, importantly corticosteroid hormones (CSs), influence the timing and nature of post-embryonic development. Stressors or treatments with CSs delay or accelerate metamorphic change, depending on the developmental stage of treatment. Also, TH and CSs have synergistic, antagonistic, and independent effects on gene regulation. Importantly, the identity of the endogenous corticosteroid hormone or receptor underlying any gene induction or remodeling event has not been determined. Levels of both CSs, corticosterone and aldosterone, peak at metamorphic climax, and the corticosteroid receptors, glucocorticoid and mineralocorticoid receptors, have wide expression distribution among tadpole tissues. Conclusive experiments to identify the endogenous players have been elusive due to difficulties in experimental control of corticosteroid production and signaling. Current data are consistent with the hypothesis that the two CSs and their receptors serve largely overlapping functions in regulating metamorphosis and synergy with TH. Knowledge of the endogenous players is critical to understanding the basic mechanisms and significance of corticosteroid action in regulating post-embryonic development in environmental contexts.

1. Introduction

Corticosteroid hormones (CSs) are highly conserved hormonal mediators of the stress response across vertebrates (Ballard, 1986; Nesan and Vijayan, 2013). CSs are also critical vertebrate developmental hormones regulating (1) organ maturation in brain, lungs, pancreas, and other organs and tissues, (2) developmental transitions of metamorphosis in fish and amphibians, hatching in chicks, and birth in mammals, and (3) long-term effects of stress during development, such as survival and fecundity in frogs, growth and coping styles in birds, and cardiovascular and metabolic health in humans (Braun et al., 2013; Crino et al., 2014; Hu et al., 2008; Nesan and Vijayan, 2013; Schoech et al., 2012) These corticosteroid-dependent developmental processes and their interactions with the environment are complex, and the signaling mechanisms and downstream effectors underlying the effects of stressors on development are largely unknown.

Progress to identify the endogenous players of corticosteroid action in development has been made using knockout animals lacking CSs or their receptors in mouse and zebrafish. Glucocorticoid receptor knockout mice and offspring of mice deficient in adrenocorticotropin (pituitary hormone required for glucocorticoid synthesis) all die just after birth due to lung atelectasis from complete lack of glucocorticoid signaling (Cole et al., 2001; Saedler and Hochgeschwender, 2011). Lack of aldosterone synthase results in 70% survival after weaning (Lee et al., 2005), but MR knockout mice all die of salt wasting around weaning (Bleich et al., 1999). Even though the acute causes of death are known and the survivors serve as good models for studying adult deficiencies, the numerous pleiotropic actions of these hormones affecting various aspects of fetal development are understudied. Embryonic effects of glucocorticoid receptor have been studied in more detail in fish. Knockdown of glucocorticoid receptor translation by morpholinos showed internal and external morphological defects in zebrafish (Nesan et al., 2012; Pikulkaew et al., 2011), as well as impaired ionocyte development in medaka (Trayer et al., 2013). Genetic disruption of adrenocorticotropin identified roles for this hormone and its receptor in the development of interrenal tissue (To et al., 2007).

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Genetic manipulations of genes involved in corticosteroid signaling is advanced in mouse and fish compared to tadpoles. However, fetal endocrine manipulations used to elucidate developmental roles of hormones are often challenging because of confounding maternal influences in mice. Also, potential developmental roles of mineralocorticoids are not possible in fish, due to their lack this class of steroid. Frog metamorphosis has been a leading model in developmental endocrinology (Shi, 2009) due to the complete dependence of dramatic morphological changes on thyroid hormone and the occurrence of free-living tadpoles rather than uterus-enclosed embryos. In addition, tadpole growth and development are especially sensitive to environmental stressors, which include climate change, endocrine disruption, and habitat disturbance (Hayes et al., 2010). On the other hand, fundamental aspects of corticosteroid physiology in development have not been elucidated in frogs.

Most metamorphosis research has focused on the role of thyroid hormone (TH) because it is necessary and sufficient to initiate metamorphic events (Brown and Cai, 2007; Dodd and Dodd, 1976; Shi, 1999). The involvement of CSs in metamorphosis is more complex than TH, and understandably, the roles of CSs and their receptors on gene regulation and metamorphic transformation are less clear. The main CSs produced by the interrenals in frogs are the glucocorticoid corticosterone (CORT, the frog stress hormone) and aldosterone (ALDO, the frog mineralocorticoid hormone) as determined by *in vitro* biosynthesis studies in tadpoles and adults of *Lithobates catesbianus* and *Xenopus laevis* (Carstensen et al., 1961; Chan and Edwards, 1970; Jolivet-Jaudet and Leloup-Hatey, 1984; Ulick and Solomon, 1960) Previous excellent reviews have included sections on corticosteroids in metamorphosis (Denver, 2009; Denver, 2013; Dodd and Dodd, 1976; Kaltenbach, 1996; Wada, 2008; White and Nicoll, 1981). The current review outlines the central control of corticosteroid production and effects of CSs on metamorphosis and then critically examines the knowns and unknowns concerning the endogenous hormones and receptors involved in corticosteroid signaling regulating metamorphic transformation.

2. Hypothalamus–pituitary–interrenal and hypothalamus–pituitary–thyroid axes

Metamorphosis begins when TH first enters circulation, causing premetamorphic tadpoles to enter prometamorphosis (Etkin, 1964; Leloup and Buscaglia, 1977). When circulating levels of TH and CSs reach their peak, tadpoles experience metamorphic climax, a period of dramatic morphological remodeling and physiological changes. TH levels return to baseline at the end of metamorphosis upon complete tail resorption. Brain processing of external environmental signals from predators, water availability, food, temperature as well as internal signals such as energy balance determines the levels of TH and CSs produced by the hypothalamus–pituitary–interrenal and hypothalamus–pituitary–thyroid axes and thus determines the timing of and size at metamorphosis (Denver et al., 2009). Synthesis and release of TH by the thyroid glands and CSs by interrenal glands (homologous to mammalian adrenal glands) are induced by the pituitary hormones thyrotropin (thyroid stimulating hormone, TSH) and corticotropin (adrenocorticotropic hormone, ACTH), respectively (Denver et al., 2009). The release of these pituitary hormones is under the influence of the hypothalamus. In adult frogs, thyrotropin releasing hormone (TRH) stimulates TSH release (Darras and Kuhn, 1982), and corticotropin releasing hormone (CRH) stimulates the release of TSH and ACTH (Kuhn et al., 1998; Tonon et al., 1986). In tadpoles and axolotls, CRH regulates the release of both TSH and ACTH, but TRH control over TSH release develops only after metamorphic climax (Denver, 1996; Jacobs et al., 1988; Kühn et al., 2005) TH exerts negative feedback on the hypothalamus–pituitary–thyroid axis throughout the larval period (Manzon and Denver, 2004), but negative feedback by CSs on CRH or ACTH has not been shown in tadpoles.

3. Effects of CSs on metamorphic progression

Early reports revealed that CSs (both CORT and ALDO) accelerate TH-induced metamorphic changes in tadpoles but have no metamorphic effect in the absence of TH in *Bufo bufo*, *Babelomurex japonicus*, and *X. laevis* (Bock, 1938; Frieden and Naile, 1955; Gasche, 1945; Kobayashi, 1958). Sub-epidermal implantation of cortisol or desoxycorticosterone acetate (a mineralocorticoid) pellets caused local tail resorption only in the vicinity of the pellet if sub-threshold doses of TH were also included in the pellet in *Lithobates pipiens* (Kaltenbach, 1958). Later reports showed that TH induction of tail shrinkage, limb outgrowth, head shape change, gut tube shortening, skin keratin expression, and hepatic enzyme carbamoyl-phosphate synthase activity were increased upon co-treatment with CORT in *X. laevis* (Galton, 1990; Gray and Janssens, 1990; Shimizu-Nishikawa and Miller, 1992; Wright et al., 1994). *In vitro* studies on cultured tail tips showed CORT and ALDO accelerated TH-induced tail shrinkage in *B. japonicus* and *X. laevis* (Bonett et al., 2010; Gray and Janssens, 1990; Kikuyama et al., 1983). In contrast, during premetamorphosis (when endogenous TH is low or absent), exogenous treatment with CORT, cortisol, dexamethasone (a glucocorticoid receptor-specific agonist), ALDO, and desoxycorticosterone acetate inhibited growth and development in *Anaxyrus boreas*, *B. japonicus*, and *X. laevis* (Hayes, 1995; Kobayashi, 1958; Leloup-hatey et al., 1990; Lorenz et al., 2009; Rapola, 1962; Wright et al., 1994) In prometamorphosis (circulating TH present), treatment with CORT or desoxycorticosterone acetate alone increased metamorphic rate in *A. boreas* and *B. japonicus* (Hayes, 1995; Kobayashi, 1958). In *X. laevis*, CORT treatment during prometamorphosis still blocked TH-induced tail resorption and forelimb emergence, though gill resorption still occurred, and ALDO had no effect (Leloup-Hatey et al., 1990). In the CORT-treated tadpoles, TH levels in plasma declined consistent with inhibition of metamorphosis. Rearing conditions that induce stress and increase CORT content during prometamorphosis resulted in an increased rate of metamorphosis in spadefoot toads (Denver, 1998; Kulkarni et al., 2011; Newman, 1989). In summary, exogenous treatment of the two classes of CSs (glucocorticoid and mineralocorticoid) have generally comparable effects on growth and development in tadpoles.

Despite clear effects on growth and development, exogenous hormone treatments per se do not reveal the endogenous actors of corticosteroid physiology. Experiments blocking corticosteroid signaling are required to elucidate such roles. Hypophysectomized (pituitary removed) tadpoles of *Alytes obstetricans* did not undergo metamorphosis (for lack of pituitary signal to make TH) but did initiate metamorphosis upon TH treatment. However, the tadpoles were unable to complete metamorphosis, unless ACTH was also given (Remy and Bounhiol, 1971). Tadpoles or tail tips treated with amphenone B (glucocorticoid synthesis inhibitor) showed reduced rate of induced metamorphosis (Kikuyama et al., 1982). Treating *A. boreas* tadpoles with metyrapone (another glucocorticoid synthesis inhibitor) caused 33% reduction in CORT, which resulted in reduced rate of hindlimb development but did not affect the rate of tail resorption (Hayes and Wu, 1995). Further work by Glennemeier and Denver showed that treatment with metyrapone reduced whole body CORT by 50% in *Lithobates pipiens* tadpoles but did not affect the rate of metamorphosis (Glennemeier and Denver, 2002b). ALDO was not measured even though the inhibitors also block aldosterone synthase activity. These experiments

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