Comparative endocrinology in the 21st century

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Synopsis Hormones coordinate developmental, physiological, and behavioral processes within and between all living organisms. They orchestrate and shape organogenesis from early in development, regulate the acquisition, assimilation, and utilization of nutrients to support growth and metabolism, control gamete production and sexual behavior, mediate organismal responses to environmental change, and allow for communication of information between organisms. Genes that code for hormones; the enzymes that synthesize, metabolize, and transport hormones; and hormone receptors are important targets for natural selection, and variation in their expression and function is a major driving force for the evolution of morphology and life history. Hormones coordinate physiology and behavior of populations of organisms, and thus play key roles in determining the structure of populations, communities, and ecosystems. The field of endocrinology is concerned with the study of hormones and their actions. This field is rooted in the comparative study of hormones in diverse species, which has provided the foundation for the modern fields of evolutionary, environmental, and biomedical endocrinology. Comparative endocrinologists work at the cutting edge of the life sciences. They identify new hormones, hormone receptors and mechanisms of hormone action applicable to diverse species, including humans; study the impact of habitat destruction, pollution, and climatic change on populations of organisms; establish novel model systems for studying hormones and their functions; and develop new genetic strains and husbandry practices for efficient production of animal protein. While the model system approach has dominated biomedical research in recent years, and has provided extraordinary insight into many basic cellular and molecular processes, this approach is limited to investigating a small minority of organisms. Animals exhibit tremendous diversity in form and function, life-history strategies, and responses to the environment. A major challenge for life scientists in the 21st century is to understand how a changing environment impacts all life on earth. A full understanding of the capabilities of organisms to respond to environmental variation, and the resilience of organisms challenged by environmental changes and extremes, is necessary for understanding the impact of pollution and climatic change on the viability of populations. Comparative endocrinologists have a key role to play in these efforts.

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The science of chemical mediation

Chemical mediators signal between cells within an organism, or between organisms, and function by binding to proteins expressed on the surface of, or within, target cells to elicit a change in cell physiology. They first evolved in single-celled organisms for communiation among individuals (e.g. quorum sensing in bacteria; Miller and Bassler, 2001). In metazoans, they play pivotal roles in coordinating development, physiology, and behavior, and the interactions among individuals within populations and communities. Chemical mediators influence how individuals develop, function, and interact with their environment, but they also underlie population-level responses to environmental change. Knowledge of these actions is essential to understand and predict how habitat fragmentation, environmental contamination by industrially-derived compounds, and climatic change may impact the viability of populations, communities, and ecosystems.

The general term ''chemical mediator'', as used here, encompasses several terms that are used within specific scientific disciplines. Hormones, the focus of the science of endocrinology, are a type of chemical mediators, originally defined as organic chemicals that are released from living cells into the blood or interstitial fluid and that travel via the bloodstream some distance from their site of production to target tissues where they coordinate physiological processes (Gorbman and Bern, 1962). As our knowledge of hormones and hormone receptors increases, the definition of a hormone continues to evolve. For example, the classical view of a hormone has changed in recent years to include actions on the cell producing the hormone (autocrine), on adjacent cells within tissues through cell–cell communication (paracrine), or on other individuals of the same or a different species (ectocrine). Chemical mediators acting as hormones are often distinguished from neurotransmitters in that the latter are released at synapses to allow for propagation of electrical signals among nerve cells, and hormones and neurotransmitters are distinguished from cytokines which function in cell-to-cell signaling in defense of the body against invading pathogens. Despite these operational definitions that distinguish among hormones, neurotransmitters, and cytokines, there are many examples in which a chemical functions in more than one role; i.e. a hormone may also function as a neurotransmitter, and vice versa. Furthermore, the endocrine, nervous, and immune systems interact at many levels in the maintenance of homeostasis and survival.

The comparative study of hormones in diverse species dates to the early part of the 20th century, when the field of endocrinology first developed. Prior to 1940, research in endocrinology was almost exclusively associated with medical schools (Kobayashi, 1983). The comparative study of animal hormones developed with the expansion of the field of Zoology during the 1940s and 1950s, and the formal discipline of comparative endocrinology was ''born'' in 1954 with the First International Symposium on Comparative Endocrinology, held at Liverpool, England. Recent decades have brought startling advances in our understanding of animal hormones and their actions, in large part due to advances in biochemistry, molecular biology, and genetics. Hundreds of hormones have now been identified, and new medical therapies, means for enhancing the production of animal protein for food, and strategies for biological control have emerged. The fields of comparative endocrinology and biomedical endocrinology continue to be closely associated, and because hormones play central roles in so many aspects of life, endocrinologists will continue to make seminal contributions that impact all disciplines of the life sciences. In this review, we discuss some of the major contributions that comparative endocrinologists have made to the science of endocrinology, and we highlight the emerging areas of research and how endocrinologists can contribute to the study of organismal biology in the 21st century. Comparative endocrinology has a rich history, and cutting-edge research in the field is now being conducted in laboratories throughout the world.

Structure and function of animal endocrine systems—the contributions of comparative endocrinologists to biomedical research

In 1849, Arnold Adolph Berthold of the University of Göttingen reported the first endocrinological experiment in which he castrated cockerels and found that this caused regression of secondary sex characters, such as the wattles and comb, and the loss of male-typical sexual behavior (Berthold, 1849). The term ''hormone'' was first coined by Ernest Starling, who together with his brother-in-law, William Bayliss, found that the upper part of the dog's small intestine, the duodenum, produced a substance (secretin) that caused secretion of pancreatic juice into the small intestine. This was the first demonstration that factors transported via the bloodstream could act on other tissues and coordinate physiological functions (Henderson, 2005). In the 19th century, the role of the pituitary gland in growth of the body was suggested by post-mortem observations of humans that suffered from acromegaly, but the first experimental evidence that the pituitary produced a substance that influenced bodily functions was the discovery made in the early 20th century that pituitary extract caused growth of the gonads of frogs (Greep, 1988).

The discovery of neurosecretion and neuropeptides marked a revolution in physiology, which led to the integration of endocrinology, neurobiology and behavior, and later immunology. Comparative studies played a pivotal role in the development of the concept of neurosecretion and of the field of neuroendocrinology. The earliest work on neurosecretion and neurohemal structures was carried out in insects (see Kopec, 1922). Ernst and Berta Sharrer, and Wolfgang Bargmann, working from the 1930s to the 1960s,

are credited with having established the intellectual basis for the field of neuroendocrinology. Ernst Sharrer first developed the concept of neurosecretion based on his work with the minnow, *Phoxinus laevis*, in which he postulated that specific neurons in the preoptic nucleus of the hypothalamus possessed endocrine activity related to pituitary function (Klavdieva, 1995). He and his wife Berta conducted comparative studies on animal neurosecretory systems, dividing their efforts, with Berta studying invertebrates (Scharrer, 1941) and Ernst studying vertebrates (Scharrer and Scharrer, 1937). Wolfgang Bargmann is credited with having firmly established the existence and functional role of neurosecretion in vertebrates (Klavdieva, 1995). Bebnado Houssay, working with toads, was the first to show that blood flowed from the hypothalamus to the pituitary gland (Houssay et al. 1935a, 1935b), and Geoffrey Harris later showed, in studies conducted with rats, that the functioning of the nervous and the endocrine systems were linked through neurohormones produced in the hypothalamus that controlled pituitary hormone secretion (Harris and Jacobsohn, 1952).

The contribution of comparative studies to the field of neuroendocrinology continues today. Many new neuropeptides were originally discovered in invertebrates and nonmammalian vertebrates, and their orthologs were subsequently found in mammals. For example, a cardioexcitatory peptide with a characterisitic FMRFamide C-terminal sequence was first isolated in 1977 by Price and Greenberg from the ganglia of the clam, *Macrocallista nimbosa* (Price and Greenberg, 1977). Recently, RFamide peptides were discovered in mammals and found to play critical roles in controlling pituitary hormone secretion, reproduction, appetite and pain, among other functions (Chartrel et al. 2003; Fukusumi et al. 2006; Tsutsui, 2009). The concentration of neuropeptides in the frog brain is estimated to be an order of magnitude greater than that of mammalian brain, which has facilitated the discovery of novel vertebrate neuropeptides (Chartrel et al. 2006).

There are many examples of neuropeptides and neuropeptide actions first discovered in nonmammalian species that were subsequently found to play important roles in human physiology and disease states. For example, the neuropeptide arginine vasotocin (the mammalian homolog is arginine vasopressin–AVP) was first found to influence reproductive behavior in amphibians, and is now known to control social behavior in diverse vertebrate species (Goodson and Bass, 2001). Recent discoveries implicate AVP in human pair-bonding behavior (Walum et al. 2008), and mental health disorders such as autism (Wassink et al. 2004; Egashira et al. 2007). The isolation of urotensin peptides from the fish caudal neurosecretory system (the urophysis) is another example of how comparative endocrinology has laid the foundation for understanding human physiology. The recently discovered human homolog of fish urotensin II is now implicated in human cardiovascular function and heart disease, and may also function as a neurotransmitter/neuromodulator in the brain (Maguire and Davenport, 2002).

The nuclear mechanisms of action of steroid hormones were first discovered by comparative biologists working with insects, and these and other nonmammalian model species continue to play a central role in the study of steroid hormone action in development, physiology, and disease. Steroid hormones bind to nuclear receptors to regulate gene expression. This concept first came from studies of the insect steroid ecdysone that was found to induce "puffing" of the giant polytene chromosomes in the salivary glands of midges and flies. This phenomenon was first observed by Clever and Karlson (1960) in the midge *Chironomus* and later expanded into a theory of a transcriptional cascade of hormone action by Ashburner et al. (1974). This theory, which has had broad impact in biology and medicine, described a gene-regulation cascade directly induced by the hormone ecdysone and that led to the tissue-specific activation or suppression of genes. Work done in the early 1990s in which ecdysone target genes were cloned showed that most of the direct-response genes were transcription factors (King-Jones and Thummel, 2005). Recent studies with insect, crustacean, and amphibian nuclear hormone receptors are helping to unravel the complexities of receptor dimerization (Kozlova et al. 2009), transcriptional regulation (King-Jones and Thummel, 2005; Buchholz et al. 2006; Hopkins et al. 2008), and the roles of nuclear hormone receptors in animal development (King-Jones and Thummel, 2005; Buchholz et al. 2006).

In addition to the well-known genomic actions of steroid hormones, rapid, nongenomic actions are now known to be mediated by the receptors located in the plasma membrane. Rapid actions of steroids were first discovered in the 1970s by Godeau et al. (1978) who showed rapid, membrane-mediated effects of progesterone on frog oocyte maturation. The first discovery and pharmacological characterization of a membrane steroid receptor located in neuronal membranes was carried out in the male rough-skinned newt in which the stress hormone corticosterone causes rapid inhibition of males' clasping behavior (Orchinik et al. 1991). In 2003 Peter Thomas and colleagues, working with ovaries of the spotted sea trout, isolated and characterized the first G protein-coupled receptor (GPR) that mediates rapid steroid actions. The fish receptor was activated by progestins (Zhu et al. 2003b), and subsequently orthologous genes were identified in mice and humans (Zhu et al. 2003a). These findings have set the stage for the discovery of other GPR steroid receptors, and the expansion of the field of nongenomic steroid hormone actions.

The invertebrates have played a major role in the development of the field of comparative endocrinology, and the findings of invertebrate endocrinologists have had far-reaching impact on the life sciences as a whole. For example, studies by Michael Berridge in the 1970s on the blowfly led to the discovery of the phosphatidylinositol signaling pathway, its role in mobilization of intracellular calcium, and more generally the role of calcium in intracellular signaling

(Berridge, 1993). The role of neuropeptides acting on the central nervous system to elicit discrete behaviors was first discovered in the mollusk, *Aplysia californica*, in which egg-laying hormone was shown to act on the nervous system to elicit stereotypical oviposition behavior (Strumwasser, 1984; Smock and Arch, 1986). Another well-characterized example of hormonal control of behavior is ecdysis in insects, in which ecdysis-triggering hormone and eclosion hormone cooperate to activate neuropeptidergic pathways in the nervous system leading to ecdysis (Truman, 2005; Zitnan et al. 2007). More recently, studies of insects are leading the way in linking control of growth and body size, and its hormonal regulation, to nutrient intake and insulin signaling (Nijhout, 2003a, 2003b; Mirth and Riddiford, 2007; Shingleton et al. 2007).

The study of mammalian model organisms such as the rat and the mouse have provided extraordinary insight into the molecular and cellular mechanisms of hormone biosynthesis and action, but relying on one or a few species for research has important limitations. The model systems approach assumes that the findings from a handful of model organisms (now primarily the mouse) can be extrapolated broadly to other species, most importantly to humans. However, these animal models may not be ideal for some basic research questions such as the roles of hormones in development, for which invertebrate or nonmammalian vertebrate models may be better suited. Importantly, model systems cannot represent the diversity of structure and function, and life-history strategy among animals. This is of particular concern for conservation biology, in which species use different physiological and behavioral strategies to survive, and may show differential susceptibility to environmental contaminants and to environmental degradation (environmental stressors). The study of one or a few model species may not provide relevant information for the species of concern, and inbreeding of model species in the laboratory reduces interindividual variation and plasticity that are critical for population sustainability in the wild. The comparative study of animal endocrine systems can lead to the development of new model systems for biomedical research, and can provide a rational basis for the development of strategies for wildlife conservation.

Evolutionary endocrinology

Variation in Darwinian fitness results from variation in organismal form, function and life-history traits. Hormones have widespread and diverse actions in coordinating the expression of animal form and function, and are thus key players in determining fitness. Natural selection acts on genes that code for hormones, hormone synthesizing or metabolizing enzymes, hormone binding proteins and receptors, and hormone signaling pathways that influence the evolution of animal diversity. Evolutionary endocrinology is a subdiscipline of evolutionary physiology (Garland and Carter, 1994), whose broad goal is to understand the manner and mechanism by which organismal function has responded to natural selection (Zera et al. 2007). Specifically, it is the study of how animal hormones and their signaling pathways have evolved to control diverse developmental, physiological, and behavioral processes; of evolutionary relationships among animal species by comparing endocrine organs, processes and genes; and of how hormone systems underlie adaptation to diverse environments and the evolution of new traits and formation of new species.

Hormones influence virtually every morphological, physiological, and life-history trait of an animal. Understanding the physiological/endocrinological mechanisms is essential to our understanding of the mechanistic underpinnings for evolutionary correlations and constraints commonly observed at higher levels of biological organization (e.g. animal form and physiological performance) (Husak et al. 2009). The actions of hormones represent a complex network of interactions, and selection may act at any point within these networks. Hormones mediate trade-offs among life-history traits (e.g. development versus growth; growth versus reproduction), the interactions between the environment and genes, and the establishment of constraints on phenotypic expression (the range and limits of phenotypic plasticity) and phenotypic evolution (e.g. maximum, species-specific body size or allometric relationships among organs/body structures). Hormones also play a key roles in the evolution of development (e.g. heterochrony developmental plasticity, polyphenisms) and the evolution of life histories (e.g. timing of metamorphosis or birth, survivorship, age at first reproduction, clutch or litter size, and frequency of reproductive cycles) (Zera et al. 2007).

Variation in endocrine function underlies variation in animal morphologies and life-history patterns. For example, components of thyroid physiology determine variation in metamorphic timing among frog species, and this timing correlates with the relative permanence of the larval habitat (Buchholz and Hayes, 2005). Thus, evolution of the length of the larval period, which is a central amphibian life-history trait, is governed by changes in the endocrine system that controls metamorphosis. The evolution of paedomorphic life histories among salamander species likely depended, in part, on mutations in genes that control production or action of thyroid hormone (Voss et al. 2000; Voss et al. 2003; Safi et al. 2006). Size-dependent, photoperiodic stimulation of growth hormone and cortisol both control development of salinity tolerance that occurs during downstream migration of juvenile salmon, and this endocrine response is reduced in landlocked salmon that have abandoned seaward migration. (McCormick, 2009). These examples show how the study of hormone-dependent phenomena in a developmental and ecological context can contribute to an understanding of the mechanistic basis for the evolution of animal diversity, and provide an intellectual basis for the development of a subfield of comparative endocrinology, evolutionary developmental endocrinology.

Variation in nucleotide sequence in hormone and hormone-receptor genes are linked to developmental, physiological, morphological, and behavioral diversity among species. For example, changes in the melanocortin receptor type 1 (MC1R) gene, which mediates actions of hormones, such as α-MSH, on pigmentation, are linked to variation in melanin-based, dark plumage color in birds (Mundy, 2005; Pointer and Mundy, 2008), and in coat color in mammals (Nachman et al. 2003). Changes in the coding sequence of the MC1R underlie the evolution of pigmentation loss in cave-dwelling fish (Gross et al. 2009). Interestingly, the de-pigmented phenotype has arisen independently in geographically separate caves through different mutations of the MC1R. Genes like the MC1R, and other hormone or hormone-receptor genes, may be frequent targets for mutation in the repeated evolution of similar phenotypes, owing to the central roles they play in development, physiology, and morphology. Insulin-like growth factor 1 (IGF-1) plays a key role in controlling body growth, and variation in the IGF-1 gene is linked to variation in body size in dogs, suggesting that this locus is a target for both artificial and natural selection (Sutter et al. 2007). The scaling of body parts (allometric scaling) is a fundamental feature of animal form and function, and findings in insects point to a key role for insulin/IGF signaling in controlling allometric relationships among body parts (i.e. body shape; Emlen et al. 2006; Shingleton et al. 2007). Recent work in invertebrates and vertebrates implicate insulin/IGF signaling in the control and evolution of lifespan (Partridge, 2008).

Hormones are key mediators of phenotypic plasticity (the property of individual genotypes to produce different phenotypes under different environmental conditions) (Pigliucci, 2001). Phenotypic plasticity may be an important driver of evolutionary change (e.g. through genetic assimilation) (Pigliucci et al. 2006), and may influence the evolution of animal life histories. For example, the neurohormone arginine vasotocin (AVT) causes shifts in sex-typical behavior in reef fish that change sex (Semsar and Godwin, 2003), and in behavioral diversification in pupfishes found in Death Valley (Lema, 2006, 2008). The neural/neuroendocrine pathways in which AVT functions as a neurotransmitter, and that mediate sex-typical behaviors, show plasticity in response to a changing social environment (Semsar and Godwin, 2003; Lema, 2006, 2008). Steroid hormones play central roles in sexual and stress-related behaviors, and modulation of their production and actions plays a key role in behavioral plasticity and in the evolution of behavioral modes and social structures (Adkins-Regan, 2005). Neurohormones of the corticotropin-releasing hormone family mediate environmental effects on the timing of amphibian metamorphosis and on the timing of birth in mammals (Denver, 2009). These are just a few examples of the many ways in which hormones mediate environmental effects on development, physiology, and behavior and provide the mechanistic basis for the evolution of diversity in morphology and life history.

The application of molecular biology to the function and evolution of the endocrine system has revolutionized comparative and evolutionary endocrinology. The mapping of genomes from species in key phylogenetic positions, such as the cephalochordate amphioxus (*Branchiostoma floridae)*, the urochordate sea squirt (*Ciona intestinalis)*, and the vertebrate sea lamprey (*Petromyzon marinus)*, is allowing comparative endocrinologists to understand the evolutionary history of vertebrate endocrine systems at the molecular level (Sherwood et al. 2005; Holland et al. 2008; Kavanaugh et al. 2008; Paris et al. 2008; Sower et al. 2009; Tello and Sherwood, 2009). Molecular phylogenetic analyses of the neurohypophysial nonpeptides (Acher et al. 1997), gonadotropin-releasing hormone (Kavanaugh et al. 2008; Okubo and Nagahama, 2008; Tsai and Zhang, 2008; Sower et al. 2009), and proopiomelanocortin (Dores and Lecaude, 2005), to name just a few, have helped to clarify phylogenetic relationships, structure/function associations, and the evolution of diversity in physiological control.

The nuclear receptor superfamily evolved over 500 million years ago, and represents a fascinating case study of molecular evolution. Joe Thornton and colleagues used phylogenetic reconstruction to ''resurrect'' the predicted ancestral steroid receptors, and then they tested the functional characteristics of these receptors using techniques of modern molecular endocrinology. This allowed the discovery that the ancestral steroid receptor of vertebrates was an estrogen receptor-like protein that first evolved in invertebrates (Thornton, 2001; Thornton et al. 2003).

Environmental endocrinology, global change, and conservation

One of the greatest challenges to biologists in the 21st century is to understand the molecular and cellular mechanisms underlying how organisms perceive environmental change, and then transduce that information into neural and neuroendocrine secretions that orchestrate morphological, physiological, and behavioral responses. The bewildering array of potential cues from the physical and social environments can actually be simplified into two major groups (or types). First, environmental information can be used for the predictable environment such as day and night, high tide/low tide, and the seasons. Therefore, organisms can use environmental cues to time and prepare for future events such as breeding, migration and hibernation. Hormones thus transduce predictive environmental signals, such as annual change in day length (or photoperiod), temperature, rainfall, or abundance of food into developmental, morphological, physiological, and behavioral responses. While mechanisms underlying photoperiodic responses have received extensive attention, mechanisms whereby organisms respond to other predictive environmental cues remain much less studied.

Second, organisms must respond appropriately to unpredictable events

in the environment, including potential stressors such as storms, predators, drought, and floods. In recent decades, human disturbance (loss of habitat, urbanization, pollution, recreational disturbance, invasive species, and spread of disease) has exacerbated how animals cope with the unpredictable environment (e.g. Travis, 2003). In contrast to hormonal responses to the predictable life cycle, animals must respond to unpredictable events during, or very soon after, the perturbation. This is a fundamentally different suite of mechanisms from responses to the predictable. Thus, although hormones mediate the interaction between the environment and the genotype, the mechanisms involved can be very different depending upon context and predictability. Understanding these two major types of response to the environment, and interactions between them, is crucial for an understanding of how, and whether, organisms will cope with global change (Wingfield, 2008).

Endocrine disruption

Disruption of hormone signaling by industrially derived chemicals [endocrine disrupter compounds (EDCs)] may compromise organismal function, and is now recognized as a significant threat to the health of human and wildlife populations. A recent position paper published by the Endocrine Society highlights the growing evidence and concern for EDC impacts on humans and wildlife (Diamanti-Kandarakis et al. 2009). The potential for endocrine disruption was first recognized when wildlife populations began to experience reproductive problems; e.g. decline of the bald eagle population on the Gulf Coast of Florida in the late 1940s, of the river-otter population in England in the 1950s, of the herring-gull population of Lake Ontario, of the western-gull population of the Channel Islands of California in the 1970s, and population decline and male reproductive deformities in alligators living in Lake Apopka, Florida, in the 1980s, as well as limb deformities and altered sex ratios of frogs in the 1990s (Colburn et al. 1996; Guillette and Guillette, 1996; Taylor et al. 2005; Hogan et al. 2008; Iguchi and Katsu, 2008). Some of the earliest indications that chemicals in the environment could mimic endogenous hormones came from studies of invertebrates in the 1960s and 1970s that showed that chemicals derived from newspaper could mimic insect juvenile hormone (Slama and Williams, 1966) and that water-borne chemicals could disrupt crustacean life cycles (Bookhout and Costlow, 1970). The study by Slama and Williams (1966) led to the development of Insect Growth Regulators (hormonal mimics) for the selective control of insect pests (Dhadialla et al. 1998). In the 1980s, several investigators discovered that tributyltin from marine paints acts as a hormonal mimic that induces intersexes (imposexes) in mollusks (Spence et al. 1990; Alzieu, 1998). Numerous studies of vertebrate wildlife and experimental animals have since shown that EDCs can have estrogenic, anti-androgenic, and anti-thyroid effects (Diamanti-Kandarakis et al. 2009). In 2008, the United States Environmental Protection Agency (EPA) established an Endocrine Disrupter Screening Program comprised of a battery of tests to evaluate the potential for industrially derived chemicals to alter androgen, estrogen, or thyroid-hormone signaling (US EPA, 2008). Several of the assays use nonmammalian species (e.g. the amphibian metamorphosis assay; the fish reproduction assay) for which knowledge of the biology and endocrinology of these animals was derived from basic research conducted by comparative endocrinologists, and upon which future development of these and other assays will depend.

While some populations, or individuals within populations appear to be unaffected by EDCs, others may be much more sensitive and show increased mortality or reduced reproductive success (e.g. Norris, 2000; Norris, 2006). What are the mechanisms for these differences? In addition to a need for knowledge of the molecular and physiological pathways that are altered by EDCs, much more information is needed concerning the effects of endocrine disrupting chemicals on free-living populations in which subtle effects on development, physiology and behavior may have far reaching, long-term effects not necessarily apparent from studies of captive animals. Basic knowledge of how animals perceive and transduce environmental information will thus be fundamental to understanding how, and whether, they can cope with EDCs (Wingfield and Mukai, 2009).

Conservation endocrinology

Endocrinologists can make significant contributions to conservation biology by helping to understand the mechanisms by which organisms cope with changing environments. In recent years physiologists and endocrinologists have provided approaches to address conservation issues relevant to land managers who make decisions on how to conserve habitat as well as protect specific populations (e.g. Cockrem, 2005; Wikelski and Cooke, 2006). For example, a given population may be impacted by environmental stress, which can often be detected by measuring a number of endocrine-related endpoints (Cyr and Romero, 2009). Endocrine biomarkers may also be useful in detecting EDCs and other lethal and sublethal contaminants. Alternatively, changes in the environment such as climate change may lead to inappropriate timing of endocrine-controlled life history events, the phenology of which can be determined by examining altered patterns of circulating hormones. Field endocrine techniques can provide substantial information on the growth, stress and reproductive status of individual animals, thereby providing insight into current and future responses of populations to changes in the environment. In addition, basic information on the environmental requirements of individual species for normal growth and development will provide critical information for species and ecosystem conservation.

Comparative endocrinology and food in the 21st century

Hormones are critical control elements of growth and reproduction and have long been targeted to increase animal food production. Knowledge of the endocrine control of growth, fat content, and appetitive behavior has led to improvements in husbandry methods in many species. Sex steroids are used to increase protein and to decrease fat content in most of the beef production in the USA (Raloff, 2002). Since 1994, growth hormone has been approved and widely used in the USA to improve milk yield in cattle. Gonadotropin releasing hormone is widely used to induce mating and spawning in many cultured fish species, especially when initial domestication is occurring (Mylonas and Zohar, 2000). Growth hormone-transgenic salmon have increased growth rates and conversion efficiency (Devlin et al. 2000). Thus, there is an opportunity for use of hormone supplements and transgenic animals to increase the efficiency, total productivity and profitability of farming operations.

There are also potential negative effects of these approaches. Hormone treatments can alter the composition of food destined for human consumption, such as the IGF-I content of milk or steroid content of meat, with possible impacts on human health. Animals themselves may be negatively influenced by hormone treatments; growth hormone treatment of cattle has been shown to result in increased mastitis, infertility, and lameness (Dohoo et al. 2003). Broader environmental impacts are also of concern. Natural and synthetic hormones may be released into the soils and waterways from concentrated animal-feeding operations (CAFOs) with potential impact on animal and human health (Jensen et al. 2006). Inadvertent release of transgenic animals could result in their interaction, including breeding, with wild animals (Muir and Howard, 2002). Both the reality and perception of these impacts has the capacity to influence consumers' responses and eventual acceptability of these treatments. Research by comparative endocrinologists can contribute in a substantive way to providing information and the clarity necessary for decisions on these trade-offs.

Domestication of new species, especially in aquaculture, can bring protein production to areas with otherwise limited production capacity and reduce pressure to harvest natural populations. Knowledge of the basic environmental requirements for the proper endocrine control of growth and reproduction will be critical for rearing of these newly domesticated species and for improving traditional approaches to husbandry of established species. Innovative techniques in animal husbandry, such as the use of altered photoperiod to improve animal growth or the timing or reproduction, are often determined or enlightened by previous understanding of basic endocrinology (Bjornsson, 1997). Hormones and receptors can act as endpoints for selection of desirable traits (Oksbjerg et al. 2004). There is increasing interest in understanding and promoting animal welfare in farming, and endocrinology can contribute by determining the factors that impose stress or compromise the health of domesticated animals.

Frontiers in comparative endocrinology

The scope of comparative endocrinology has expanded dramatically since its formal origins over 50 years ago. Studies of hormones and their actions impact virtually every field of the life sciences, and the importance of work by comparative endocrinologists for the study of organismal biology in the 21st century will only continue to increase. The neuroendocrine system transduces environmental signals into developmental, physiological, and behavioral responses, and knowledge of these mechanisms is essential for understanding how organisms interact with their environment and how the environment influences organismal form, function, and survival. Some important unanswered questions include: By what sensory modalities do organisms perceive environmental change? How is this sensory information transduced into neuroendocrine and endocrine secretions (stimulatory and inhibitory)? In what ways will global change (climate and human disturbance) affect organisms in relation to their ''perception/transduction systems?'' For example, an organism whose life cycle is driven by photoperiod may become mismatched with other changes in its environment (e.g., global warming); whereas, other organisms that respond to multiple environmental signals such as temperature and photoperiod will be more likely to adjust. What is the potential for new and existing chemicals to affect neuroendocrine systems? Why are some individuals or species less susceptible to the impacts of exposure to EDCs while others are greatly affected? Can we develop sensitive, high-throughput assays for EDCs that will be representative of endocrine disruption in a broad range of species? Only comparative studies of diverse species will allow us to address such questions.

Comparative endocrinologists have important roles to play in many areas of the life sciences, such as the development of alternative animal model systems for discovery of novel hormones and hormone-signaling pathways; the discovery of new pharmaceuticals to treat human disease; the design of hormonally-based strategies for pest control; the development of sensitive, representative and high-throughput endocrine-screening assays for EDCs; the analysis of the impact of global climatic change on animal populations; the elucidation of pathways and mechanisms of evolution through the study of endocrine genes and structures; and the development of more efficient means for the production of animal protein to feed the world's growing human population. This is not intended to be a comprehensive list, or to limit research in this field, but rather to serve as a stimulus for further thought and discussion. Critical to these efforts is the recruitment and broad training of young

scientists, continued and expanded support for their research, and the coordination of efforts among scientists in diverse areas of the life sciences who have a common interest in chemical mediation.

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References

Acher R, Chauvet J, Chauvet MT, Michel G, Rouille Y. 1997. Molecular evolution of neurohypophysial hormones in relation to osmoregulation: the two fish options. Fish Physiol Biochem 17:325–32.

Adkins-Regan E. 2005. Hormones and Animal Social Behavior. Princeton: Princeton University Press.

Alzieu C. 1998. Tributyltin: case study of a chronic contaminant in the coastal environment. Ocean & Coastal Management 40:23–36.

Ashburner M, Chihara C, Meltzer P, Richards G. 1974. Temporal control of puffing activity in polytene chromosomes. Cold Spring Harb Symp Quant Biol 38:655–62.

Berridge MJ. 1993. Inositol trisphosphate and calcium signaling. Nature 361:315–25.

Berthold AA. 1849. Transplantation of testes. 1944. English translation by DP Quiring. Bull Hist Med 16:399–401.

Bjornsson BT. 1997. The biology of salmon growth hormone: from daylight to dominance. Fish Physiol Biochem 17:9–24.

Bookhout CG, Costlow JD. 1970. Nutritional effects of Artemia from different locations on larval development of crabs. Helgolander Wissenschaftliche Meeresuntersuchungen 20:435–42.

Buchholz DR, Hayes TB. 2005. Variation in thyroid hormone action and tissue content underlies species differences in the timing of metamorphosis in desert frogs. Evol Dev 7:458–67.

Buchholz DR, Paul BD, Fu LZ, Shi YB. 2006. Molecular and developmental analyses of thyroid hormone receptor function in *Xenopus laevis*, the African clawed frog. Gen Comp Endocrinol 145:1–19.

Chartrel N, et al. 2006. Structure and functions of the novel hypothalamic RFamide neuropeptides R-RFa and 26RFa in vertebrates. Peptides 27:1110–20.

Chartrel N, et al. 2003. Identification of 26RFa, a hypothalamic neuropeptide of the RFamide peptide family with orexigenic activity. Proc Natl Acad Sci USA 100:15247–52.

Clever U, Karlson P. 1960. Induktion von Puff-Veranderungen in den Speicheldrusenchromosomen von Chironomus-tentans durch Ecdyson. Exp Cell Res 20:623–6.

Cockrem JF. 2005. Conservation and behavioral neuroendocrinology. Horm Behav 48:492–501.

Colburn T, Dumanoski D, Myers JP. 1996. Our stolen future: are we threatening our fertility, intelligence, and survival?—a scientific detective story. New York: Dutton.

Cyr NE, Romero LM. 2009. Identifying hormonal habituation in field studies of stress. General and Comparative Endocrinology 161:295–303.

Denver RJ. 2009. Stress hormones mediate environment-genotype interactions during amphibian development. Gen Comp Endocrinol 164:20–31.

Devlin RH, Swanson P, Clarke WC, Plisetskaya E, Dickhoff W, Moriyama S, Yesaki TY, Hew CL. 2000. Seawater adaptability and hormone levels in growth-enhanced transgenic coho salmon, *Oncorhynchus kisutch*. Aquaculture 191:367–85.

Dhadialla TS, Carlson GR, Le DP. 1998. New insecticides with ecdysteroidal and juvenile hormone activity. Annu Rev Entomol 43:545–69.

Diamanti-Kandarakis E, Bourguignon J.-P, Giudice LC, Hauser R, Prins GS, Soto AM, Zoeller RT, Gore AC. 2009. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. Endocr Rev 30:293–342.

Dohoo IR, DesCoteaux L, Leslie K, Fredeen A, Shewfelt W, Preston A, Dowling P. 2003. A meta-analysis review of the effects of recombinant bovine somatotropin 2. Effects on animal health, reproductive performance, and culling. Canadian Journal of Veterinary Research-Revue Canadienne De Recherche Veterinaire 67:252–64.

Dores RM, Lecaude S. 2005. Trends in the evolution of the proopiomelanocortin gene. Gen Comp Endocrinol 142:81–93.

Egashira N, Tanoue A, Matsuda T, Koushi E, Harada S, Takano Y, Tsujimoto G, Mishima K, Iwasaki K, Fujiwara M. 2007. Impaired social interaction and reduced anxiety-related behavior in vasopressin V1a receptor knockout mice. Behav Brain Res 178:123–7.

Emlen DJ, Szafran Q, Corley LS, Dworkin I. 2006. Insulin signaling and limb-patterning: candidate pathways for the origin and evolutionary diversification of beetle 'horns'. Heredity 97:179–91.

Fukusumi S, Fujii R, Hinuma S. 2006. Recent advances in mammalian RFamide peptides: the discovery and functional analyses of PrRP, RFRPs and QRFP. Peptides f 27:1073–86.

Garland T, Carter PA. 1994. Evolutionary physiology. Annu Rev Physiol 56:579–621.

Godeau JF, Schorderetslatkine S, Hubert P, Baulieu EE. 1978. Induction of maturation in *Xenopus laevis* oocytes by a steroid linked to a polymer. Proc Natl Acad Sci USA 75:2353–7.

Goodson JL, Bass AH. 2001. Social behavior functions and related anatomical characteristics of vasotocin/vasopressin systems in vertebrates. Brain Res Rev 35:246–65.

Gorbman A, Bern HA. 1962. A Textbook of Comparative Endocrinology. New York: John Wiley.

Greep RO. 1998. Gonadotropins. In: McCann SM, editor. Endocrinology: people and ideas. Bethesda: American Physiological Society. p. 63–85.

Gross JB, Borowsky R, Tabin CJ. 2009. A novel role for Mc1r in the parallel evolution of depigmentation in independent populations of the cavefish *Astyanax mexicanus*. Plos Genetics 5, e1000326.

Guillette LJ, Guillette EA. 1996. Environmental contaminants and reproductive abnormalities in wildlife: implications for public health? Toxicol Ind Health 12:537–50.

Harris GW, Jacobsohn D. 1952. Functional grafts of the anterior pituitary gland. Proc R Soc Lond B Biol Sci 139:263–76.

Henderson J. 2005. Ernest Starling and 'hormones': an historical commentary. J Endocrinol 184:5–10.

Hogan NS, Duarte P, Wade MG, Lean DRS, Trudeau VL. 2008. Estrogenic exposure affects metamorphosis and alters sex ratios in the northern leopard frog (*Rana pipiens*): identifying critically vulnerable periods of development. Gen Comp Endocrinol 156:515–23.

Holland LZ, et al. 2008. The amphioxus genome illuminates vertebrate origins and cephalochordate biology. Genome Res 18:1100–11.

Hopkins PM, Durica D, Washington T. 2008. RXR isoforms and endogenous retinoids in the fiddler crab, Uca pugilator. Comp Biochem Physiol A Mol Integr Physiol 151:602–14.

Houssay BA, Biasotti A, Sammar-Tino R. 1935a. Effects of infundibulo-tuberal lesions in the toad. Rev Soc Argent Biol 11:318–30.

Houssay BA, Biasotti A, Sammartino R. 1935b. Functional modifications of the hypophysis after the infundibulo-tuberian lesions in the frog. Comptes Rendus Des Seances De La Societe De Biologie Et De Ses Filiales 120:725–7.

Husak JF, Irschick DJ, McCormick SD, Moore IT. 2009. Hormonal regulation of whole-animal performance: implications for selection. Integr Comp Biol (In press).

Iguchi T, Katsu Y. 2008. Commonality in signaling of endocrine disruption from snail to human. Bioscience 58:1061–7.

Jensen KM, Makynen EA, Kahl MD, Ankley GT. 2006. Effects of the feedlot contaminant 17 alpha-trenholone on reproductive endocrinology of the fathead minnow. Environ Sci Technol 40:3112–7.

Kavanaugh SI, Nozaki M, Sower SA. 2008. Origins of gonadotropin-releasing hormone (GnRH) in vertebrates: identification of a novel GnRH in a basal vertebrate, the sea lamprey. Endocrinology 149:3860–9.

King-Jones K, Thummel CS. 2005. Nuclear receptors—a perspective from drosophila. Nat Rev Genet 6:311–23.

Klavdieva MM. 1995. The history of neuropeptides.1. Front neuroendocrinol 16:293–321.

Kobayashi H. 1983. A view of comparative endocrinology and Gorbman, Aubrey. Am Zool 23:739–48.

Kopec S. 1922. Studies on the necessity of the brain for the inception of insect metamorphosis. Biological Bulletin 42:323–342.

Kozlova T, Lam G, Thummel CS. 2009. Drosophila DHR38 nuclear receptor is required for adult cuticle integrity at eclosion. Dev Dyn 238:701–7.

Lema S. 2008. The phenotypic plasticity of death valley's pupfish. Am Sci 96:28–36.

Lema SC. 2006. Population divergence in plasticity of the AVT system and its association with aggressive behaviors in a death valley pupfish. Horm Behav 50:183–93.

Maguire JJ, Davenport AP. 2002. Is urotensin-II the new endothelin? Br J Pharmacol 137:579–88.

McCormick SD. 2009. Evolution of the hormonal control of animal performance: insights from the seaward migration of salmon. Integr Comp Biol (In press).

Miller MB, Bassler BL. 2001. Quorum sensing in bacteria. Annu Rev Microbiol 55:165–99.

Mirth CK, Riddiford LM. 2007. Size assessment and growth control: how adult size is determined in insects. Bioessays 29:344–55.

Muir WM, Howard RD. 2002. Assessment of possible ecological risks and hazards of transgenic fish with implications for other sexually reproducing organisms. Transgenic Res 11:101–14.

Mundy NI. 2005. A window on the genetics of evolution: MC1R and plumage colouration in birds. Proc R Soc B Biol Sci 272:1633–40.

Mylonas CC, Zohar Y. 2000. Use of GnRHa-delivery systems for the control of reproduction in fish. Rev Fish Biol Fish 10:463–91.

Nachman MW, Hoekstra HE, D'Agostino SL. 2003. The genetic basis of adaptive melanism in pocket mice. Proc Natl Acad Sci USA 100:5268–73.

Nijhout H. 2003a. The control of body size in insects. Dev Biol 261:1–9.

Nijhout HF. 2003b. The control of growth. Development 130:5863–7.

Norris DO. 2000. Endocrine disrupters of the stress axis in natural populations: how can we tell? Am Zool 40:393–401.

Norris DO. 2006. Endocrine disruption. Biological Basis for Health Effects in Wildlife and Humans. New York: Oxford University Press.

Oksbjerg N, Gondret F, Vestergaard M. 2004. Basic principles of muscle development and growth in meat-producing mammals as affected by the insulin-like growth factor (IGF) system. Domest Anim Endocrinol 27:219–40.

Okubo K, Nagahama Y. 2008. Structural and functional evolution of gonadotropin-releasing hormone in vertebrates. Acta Physiol 193:3–15.

Orchinik M, Murray T, Moore F. 1991. A corticosteroid receptor in neuronal membranes. Science 252:1848–51.

Paris M, et al. 2008. Amphioxus post-embryonic development reveals the homology of chordate metamorphosis. Curr Biol 18:825–830.

Partridge L. 2008. Some highlights of research on aging with invertebrates. Aging Cell 7:605–8.

Pigliucci M. 2001. Phenotypic plasticity. Beyond nature and nurture. Baltimore: The Johns Hopkins University Press.

Pigliucci M, Murren CJ, Schlichting CD. 2006. Phenotypic plasticity and evolution by genetic assimilation. J Exp Biol 209:2362–7.

Pointer MA, Mundy NI. 2008. Testing whether macroevolution follows microevolution: are colour differences among swans (Cygnus) attributable to variation at the MC1R locus? BMC Evol Biol 8:249.

Price DA, Greenberg MJ. 1977. Structure of a molluscan cardioexcitatory neuropeptide. Science 197:670–1.

Raloff J. 2002. Hormones: Here's the Beef. Science News 161:10–2.

Safi R, et al. 2006. Pedomorphosis revisited: thyroid hormone receptors are functional in Necturus maculosus. Evol Dev 8:284–92.

Scharrer B. 1941. Endocrines in invertebrates. Physiol Rev 21: 383–409.

Scharrer E, Scharrer B. 1937. On glandular nerve cells and neurosecretory organs in invertebrates and vertebrates. Biol Rev Camb Philos Soc 12:185–216.

Semsar K, Godwin J. 2003. Social influences on the arginine vasotocin system are independent of gonads in a sex-changing fish. J Neurosci 23:4386–93.

Sherwood NM, Tello JA, Roch GJ. 2005. Neuroendocrinology of protochordates: insights from Ciona genomics. 15th International Congress of Comparative Endocrinologists. MA: Boston. p. 254–71.

Shingleton AW, Frankino WA, Flatt T, Nijhout HF, Emlen DJ. 2007. Size and shape: the developmental regulation of static allometry in insects. Bioessays 29:536–48.

Slama K, Williams CM. 1966. Paper factor as an inhibitor of embryonic development of European bug *Pyrrhocoris apterus*. Nature 210:329–30.

Smock T, Arch S. 1986. What commands egg laying in Aplysia. Behav Brain Sci 9:734–5.

Sower SA, Freamat M, Kavanaugh SI. 2009. The origins of the vertebrate hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitary-thyroid (HPT) endocrine systems: new insights from lampreys. Gen Comp Endocrinol 161:20–9.

Spence SK, Hawkins SJ, Santos RS. 1990. The mollusc thais-haemastoma an exhibitor of imposex and potential biological indicator of tributyltin pollution. Mar Ecol 11:147–56.

Strumwasser F. 1984. The structure of the commands for a neuropeptide-mediated behavior: Egg-laying in an Opisthobranch mollusk. In: Hoffmann J, Porchet M, editors. Proceedings in life sciences: biosynthesis metabolism and mode of action of invertebrate hormones. Berlin: Springer. p. 36–43.

Sutter NB, et al. 2007. A single IGF1 allele is a major determinant of small size in dogs. Science 316:112–5.

Taylor B, Skelly D, Demarchis LK, Slade MD, Galusha D, Rabinowitz PM. 2005. Proximity to pollution sources and risk of amphibian limb malformation. Environ Health Perspect 113: 1497–501.

Tello JA, Sherwood NM. 2009. Amphioxus: beginning of vertebrate and end of invertebrate type GnRH receptor lineage. Endocrinology 150:2847–56.

Thornton JW. 2001. Evolution of vertebrate steroid receptors from an ancestral estrogen receptor by ligand exploitation and serial genome expansions. Proc Natl Acad Sci USA 98:5671–6.

Thornton JW, Need E, Crews D. 2003. Resurrecting the ancestral steroid receptor: ancient origin of estrogen signaling. Science 301:1714–7.

Travis JMJ. 2003. Climate change and habitat destruction: a deadly anthropogenic cocktail. Proc R Soc Lond B Biol Sci 270:467–73.

Truman JW. 2005. Hormonal control of insect ecdysis: endocrine cascades for coordinating behavior with physiology. Vitam Horm 73:1–30.

Tsai PS, Zhang LH. 2008. The Emergence and loss of gonadotropin-releasing hormone in protostomes: orthology, phylogeny, structure, and function. Biol Reprod 79:798–805.

Tsutsui K. 2009. A new key neurohormone controlling reproduction, gonadotropin-inhibitory hormone (GnIH): Biosynthesis, mode of action and functional significance. Progress in Neurobiology 88:76–88.

US Environment Protection Agency. 2008. Endocrine Disruptor Screening Program (EDSP). Available from http://www.epa.gov/scipoly/oscpendo/index.htm.

Voss SR, Prudic KL, Oliver JC, Shaffer HB. 2003. Candidate gene analysis of metamorphic timing in ambystomatid salamanders. Mol Ecol 12:1217–23.

Voss SR, Shaffer HB, Taylor J, Safi R, Laudet V. 2000. Candidate gene analysis of thyroid hormone receptors in metamorphosing vs. nonmetamorphosing salamanders. Heredity 85:107–14.

Walum H, et al. 2008. Genetic variation in the vasopressin receptor 1a gene (AVPR1A) associates with pair-bonding behavior in humans. Proc Natl Acad Sci USA 105:14153–6.

Wassink TH, Piven J, Vieland VJ, Pietila J, Goedken RJ, Folstein SE, Sheffield VC. 2004. Examination of AVPR1a as an autism susceptibility gene. Mol Psych 9:968–72.

Wikelski M, Cooke SJ. 2006. Conservation physiology. Trends Ecol Evol 21:38–46.

Wingfield JC. 2008. Comparative endocrinology, environment and global change. Gen Comp Endocrinol 157:207–16.

Wingfield JC, Mukai M. 2009. Endocrine disruption in the context of life cycles: perception and transduction of environmental cues. Gen Comp Endocrinol 163:92–96.

Zera AJ, Harshman LG, Williams TD. 2007. Evolutionary endocrinology: the developing synthesis between endocrinology and evolutionary genetics. Annu Rev Ecol Evol Syst 38:793–817.

Zhu Y, Bond J, Thomas P. 2003a. Identification, classification, and partial characterization of genes in humans and other vertebrates homologous to a fish membrane progestin receptor. Proc Natl Acad Sci USA 100:2237–42.

Zhu Y, Rice CD, Pang YF, Pace M, Thomas P. 2003b. Cloning, expression, and characterization of a membrane progestin receptor and evidence it is an intermediary in meiotic maturation of fish oocytes. Proc Natl Acad Sci USA 100:2231–6.

Zitnan D, Kim YJ, Zitnanova I, Roller L, Adams ME. 2007. Complex steroid-peptide-receptor cascade controls insect ecdysis. Gen Comp Endocrinol 153:88–96.