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The use of noninvasive and minimally invasive methods in endocrinology for threatened mammalian species conservation

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ABSTRACT

Endocrinology is an indispensable tool in threatened species research. The study of endocrinology in threatened species not only advances knowledge of endocrine mechanism but also contributes to conservation efforts of studied species. To this end, endocrinology has been traditionally used to understand reproductive and adrenocortical endocrine axes by quantifying excreted steroid metabolites. From these studies a large body of knowledge was created that contributed to the field of endocrinology, aided conservation efforts, and created a template by which to validate and conduct this research for other species. In this regard noninvasive hormone monitoring has become a favored approach to study the basic endocrinology of wildlife species. Due to the increased understanding of endocrine physiology of threatened species, breeding rates of captive population have improved to levels allowing for reintroduction of species to restored natural ecosystems. Although these approaches are still employed, advances in biochemical, molecular, and genomic technologies are providing inroads to describe lesser known endocrine activity in threatened species. These new avenues of research will allow for growth of the field with greater depth and breadth. However, for all approaches to endocrinology, limitations on resources and access to animals will require innovation of current methodologies to permit broad application for use in threatened species research.

1. Introduction

One in five vertebrate species is threatened with extinction, with threatened categorized as vulnerable, endangered or critically endangered species (IUCN, 2013). Obtaining basic biological information on these species compliments and augments other conservation efforts such as habitat assessment and captive management. Understanding endocrine activity provides unparalleled insight to animal and species biology because hormones affect all tissues in the body. The knowledge gained from species-focused endocrine research can be directly related back to in situ and ex situ programs, but also enhances the science of endocrinology and the collective understanding of animal biology. The growth of the field of comparative endocrinology is dependent on a broadening of understanding of the various mechanisms that compose endocrinology, and with threatened species composing nearly 20% of vertebrate species, studying these species is integral to this area of study. Finally, public awareness of threatened species provides an opportunity to engage a broader audience in the discipline of endocrinology and demonstrate that knowledge acquisition can have direct impact on these populations.

The benefits of a noninvasive approach to endocrinology have been reviewed previously for avian (Goymann, 2005), amphibian (Narayan, 2013), and mammalian (Hodges et al., 2010) species. The purpose of this review is to highlight application of these approaches in research of mammals threatened with extinction, and discuss emerging technologies that may contribute to species conservation and enhance the field of comparative endocrinology. Specifically, we address the rationale for the types of endocrine investigations taken on threatened species, the challenges and limitations of studying these rare species, the standard methods and validation approaches necessary to quantify hormones in threatened mammals, and finally emerging technologies that will advance the field. Throughout the manuscript we attempted to cite material from published studies on threatened species; however, when such source material was lacking we cited material that supports the potential application of the approach to threatened species research.

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2. Basic descriptive research

Although the importance and attraction to studying threatened species is evident, the reality of studying these species is much more complicated than traditional research on model and domestic species, and even non-threatened wildlife. The challenges are numerous, however the commonality stems from the fact the species are threatened and therefore access to animals is limited. However, the limitation expands beyond numbers to achieve robust power in statistical analysis, but investigations are further limited in ability to alter housing and management conditions to conduct controlled experiments, exogenously manipulate endocrine function to determine response to or absence of a hormone, and collection of tissue samples for cellular and molecular testing and analysis. Although the approaches described in this section are typical study methodologies, not only for endocrinology but other basic sciences as well, without a firm comprehension of the species' basic endocrine activity, such experimentation may have deleterious results. This is exemplified in wild felids where contraceptives were widely used in the 1990's, but the pervasive use caused reproductive tract pathologies in a number of species (Munson, 2006; Munson et al., 2002), and subsequent use of reproductive contraceptives has since been curtailed. Therefore, working within the restrictions of studying threatened species, a wealth of information of endocrine biology via descriptive studies has been generated that not only has affected management of the species, but also added to the body of endocrine knowledge (Hesterman et al., 2008; Howell-Stephens et al., 2012; Jewgenow et al., 2006; North and Harder, 2008; Scarlata et al., 2011; Steinman et al., 2012). This review describes the common, and sometimes unique approaches employed to study threatened species.

3. Endocrine activity to study

Given the great variety of hormone roles among vertebrate, almost all aspects of endocrinology can be investigated in any species with novel findings. Practically, however, this is not a reality and the focus of most threatened species endocrine studies has been on the hypothalamo–pituitary gonadal (HPG) and adrenocortical (HPA) axes. Comprehension of these axes affords managers considerable insight into the areas of management most desirable: reproduction and stress (Pukazhenthi and Wildt, 2004). Furthermore, as whole body biology can be affected by the HPG and HPA axes, investigations of their function yields the greatest return. Thereby, determining the factors that favor reproduction and limit stress is ideal in wild or captive management. Inability to address these issues can have detrimental effects on the sustainability of a population as failure to reproduce or poor reproduction are just as detrimental to the persistence of a species as a viral outbreak.

Although HPG and HPA endocrine studies dominate threatened species research, a few wildlife studies have demonstrated that topics such as of thyroid endocrinology (Keech et al., 2010; Wasser et al., 2010), and effects of environmental toxicants on endocrine function (Guillette and Moore, 2006) have application for threatened species. In addition to these rarely broached subjects, descriptions of endocrine diseases common in other species are nary mentioned in threatened species. The lack of endocrine disease description, however, is not an oversight or that these diseases do not exist in threatened species; but rather the databases of normative hormone values to make such descriptions or diagnoses possible do not exist. To remedy these deficits in knowledge, efforts need to be made to establish species-specific baseline values for these hormones (e.g. insulin; thyroid hormones) so that proper diagnostic and treatment protocols can be developed.

Finally, rarely are endocrine studies of threatened species solely focused on hormones. Rather, endocrine data should be paired with other disciplines and measures, including environment, behavior, husbandry, nutrition, ecology, and veterinary medicine. These interdisciplinary efforts, sometimes under the umbrella of field endocrinology (Walker et al., 2005) or conservation physiology (Wikelski and Cooke, 2006), provide needed context for appreciating hormone events (e.g. estrual behaviors with increased estrogen measures), and relevance for managerial and conservation considerations (e.g. increased glucocorticoid [GC] concentrations in response to logging activities).

4. Study populations

4.1. Captive populations

With more than 1200 mammalian species threatened with extinction (IUCN, 2013), there is not a shortage of species to study; however, accessing a population to study is very limited. The degradation, fragmentation and overall loss of habitat limits observations of animals in native habitats and thereby opportunities to conduct endocrine studies in situ are few. Alternatively, captive environments offer investigators the intimacy needed to collect routine endocrine data on animals with known life histories. Additionally, major factors such as disease, predation and food search are regulated to provide a safe and healthy environment. Yet despite limiting risks in the captive environment, the removal of animals from native environments will produce different biological activity than free-ranging counterparts, such as environmental conditions and experiencing novel events. To encourage naturalistic behaviors concerted efforts have been made in zoos and other captive settings to provide animals with environments that mimic natural conditions and/or provide positive stimuli (Hoy et al., 2010; Kleiman et al., 2010). To this end, standards of diet, husbandry, care, and management are drafted for species to standardize optimal conditions to maintain a given species in captivity, with special attention paid to threatened species (Dorsey, 2013). Although care is taken to provide standardized conditions for a species, the dispersion of a species' captive population among various holding institutions prevents true uniformity in care and management, and therefore careful record taking of these conditions must be considered when collecting endocrine data on animals maintained across varying facilities.

Species persistence is absolutely dependent on reproduction. The biological link between reproduction and endocrinology is intrinsic, with most major reproductive activities, both behavioral and physiological, being driven by endocrine changes. Therefore, tracking reproductive endocrine activity emerged as the primary endocrine tool in threatened species research. Because reproduction is a component of many threatened species plans (Bowkett, 2009; Conde et al., 2013; Wildt et al., 2010), generating information about basic aspects of reproductive cycles is a necessary part of the conservation effort. Characterizing basic reproductive endocrine trends has led to the creation of several self-sustaining captive populations, the giant panda (*Ailuropoda melanoleuca*) being an example of such success. For much of the captive history of this species, very little was known about reproductive endocrinology and captive breeding was unsuccessful (Zhang et al., 2006). A thawing of political tensions permitted for an expansion of research in the late 1990's and early 2000's, including reproductive endocrine studies (Czekala et al., 1998, 2003; Kersey et al., 2010a,b; Lindburg et al., 2001; Steinman et al., 2006). The expansion of knowledge enabled the creation of artificial insemination and timed breeding protocols (Howard et al., 2006, 2008) that have facilitated the growth of the captive population to sustainable lev-

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