Quantifying Stress in Marine Mammals: Measuring Biologically Active Cortisol in Cetaceans and Pinnipeds

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LONG-TERM GOALS

This research will improve our ability to measure stress in marine mammals. Stress hormones (glucocorticoids – either cortisol or corticosterone) are easily measured in blood and are an important measure of stress. However, a large proportion of glucocorticoids are bound by a protein in blood (corticosteroid binding globulin, CBG) and do not have immediate biological effects. Immediate biological effects are best estimated by measuring "free glucocorticoid" levels (i.e. that hormone not bound by CBG). This project will improve the capacity of marine mammal researchers to measure free glucocorticoid levels.

OBJECTIVES

This project has two main objectives, both related to improving our ability to measure stress in marine mammals by measuring free glucocorticoid levels.

First, we are measuring the binding affinity of corticosteroid binding globulin (CBG) in a broad range of marine mammal species. This measure (the equilibrium dissociation constant) is species-specific and is a required measurement for our ultimate goal of calculating free glucocorticoid concentrations.

The second objective is to measure glucocorticoid binding capacity in blood samples from individual animals. CBG binding capacity varies among individuals and can vary according to sex, season, life stage, and stress history, so it is critical to measure CBG binding capacity from as diverse a group of individuals as possble.

APPROACH

Brendan Delehanty, a postdoctoral fellow at the University of Toronto will be responsible for coordinating plasma samples from participating researchers and for perfoming the laboratory work. Some aspects of the laboratory work will be performed by students or technicians under Dr. Delehanty's supervision.

Task 1: Compare separation methods for binding affinity and binding capacity assays
The assays for measuring binding affinity and capacity both require the separation of free hormone from bound hormone. This project uses two methods for separating bound from free hormone. The first is activated charcoal, which adsorbs free hormone, and the second is glass fibre filters, which bind CBG-bound hormone. The charcoal technique is inexpensive and more readily adopted by stress researchers, but the glass fibre method is assumed to be more accurate due to its ability to rapidly separate bound and free hormone. However, the glass fibre method requires expensive equipment.

We will use a subset of our plasma samples (4 or 5 species) for this comparison. By directly comparing these two methods, we will be able to document their relative costs and benefits. This sort of direct comparison has not been done before, and it will be a very useful contribution to stress physiologists.

Task 2: Measuring species-specific binding affinities
The binding affinity of CBG is required for the calculation of free GC concentrations, but binding affinity varies from species to species (Delehanty et al. 2015). Our second task is to measure the binding affinities of CBG from a wide variety of marine mammal species (see Table 1) using saturation binding assays (Delehanty et al. 2015).

Table 1. Study species and collaborations

Collaborator	Collaborating Institution	Species
Martin Haulena	Vancouver Aquarium	Beluga False killer whale Harbour porpoise White sided dolphin Harbour seals
Cory Champagne	Old Dominion University/NMMF	Bottlenose dolphin
Dan Crocker	Sonoma State University	California sea lion Weddell seal Crabeater seal Southern sea lion Australian fur seal Australian sea lion Antarctic fur seal Northern elephant seals
Dorian Houser	NMMF	Bottlenose dolphins
Pat Fair	NOAA	Bottlenose dolphins
Tracy Romano	Mystic Aquarium	Belugas Fur seals Steller sea lions Harbor seals Gray seals Harp seals

Task 3: Measuring CBG binding capacity from individual animal plasma samples
CBG binding capacity (i.e. the amount of CBG) often varies among individuals, seasons, life stages and stress history (Breuner et al. 2013). This variation can have the effect of greatly increasing or decreasing the circulating concentration of biologically active free hormone and the amount of bound hormone that can act as a reservoir available for later diffusion out of circulation (Malisch and Breuner 2010). A number of our collaborators have plasma samples obtained from individuals involved in

experimental stress studies, or from individuals that vary in age, sex, life stage, health status or season of sampling. For these individual plasma samples, we will measure the maximum corticosteroid binding capacity. We will then be able to calculate free glucocorticoid concentrations using the total glucocorticoid concentration, the CBG binding affinity, and the CBG binding capacity (Barsano and Baumann 1989). This data will contribute to our collaborators' independent research programs.

WORK COMPLETED

This project has only just received the funds (but awarded in May 2015; delays occurred because of agreement issues) . The cell harvester (the equipment required for glass fiber filtration) has been ordered. We have contacted our U.S. and Canadian collaborators and colleagues to arrange for the shipping of samples to our laboratory. The shipment of U.S. samples involves obtaining permits under both the Marine Mammal Protection Act and, in some cases, CITES. We are in the process of preparing the permit applications. Samples from our Canadian collaborator will be shipped once the cell harvester has arrived.

RESULTS

Laboratory work has not yet started as the funds have only just been awarded. Laboratory work will commence as soon as we receive our first shipment of plasma from collaborators.

IMPACT/APPLICATIONS

Measuring stress is a complex endeavor and through this project, we will generate basic data that can be used by future researchers (species-specific CBG binding affinities). In the course of collaborating with researcher measuring CBG binding capacity in individual animals, we will be evaluating the relative merits of two commonly used methods for measuring binding capacity that have not yet been directly compared.

RELATED PROJECTS

This project in capitalizing on samples obtained by other researchers. This project will provide our collaborating researchers with data on CBG binding capacity and free glucocorticoid levels from the samples that they provide to us. The projects that will benefit from this data include "Variability of Hormonal Stress Markers Collected from a Managed Dolphin Population" (PI: Dorian S. Houser, National Marine Mammal Foundation), "Pathophysiology of Stress in Wild and Managed-Care Bottlenose Dolphins" (PI: Patricia Fair, NOAA), "Variability of Hormonal Stress Markers and Stress Responses in a Large Cross-Sectional Sample of Elephant Seals" (PI: Daniel Crocker, Sonoma State University), and "Stress Hormones and Their Regulation in a Captive Dolphin Population" (PI: Cory Champagne, Old Dominion University).

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