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*Salivary cortisol in captive dolphins (*Tursiops truncatus*): a non-invasive technique*

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Abstract

Dolphins in captivity have to cope with severe changes in their environment. So far, there are few studies on the welfare of these animals under these conditions. The aim of the present study was to find if cortisol was present in the saliva of dolphins and to explore the possibility of performing serial, non-invasive cortisol assays in captive dolphins. Saliva was collected non-invasively during a month from four dolphins that had responded to previous training, in order to provide saliva samples, in two aquaria in Mexico City. In addition, serum and saliva time-matched samples were obtained in an aquarium in Nuevo Vallarta, Mexico. Cortisol concentrations in saliva and blood were measured by radioimmunoanalysis (RIA). Results show for the first time that measurable quantities of cortisol are secreted within the saliva of dolphins. Salivary cortisol measurements could be a useful tool for carrying out long-term cortisol sampling. It is far less invasive than blood-sampling and could be used, in conjunction with behavioural observations, to monitor the welfare of captive dolphins, non-invasively.

Keywords: *animal welfare, dolphin, saliva cortisol, serum cortisol, steroids, stress*

Introduction

Bottlenose dolphins (*Tursiops truncatus*) in captivity, are used for entertainment and assisted therapy in several places, however these animals have to cope with changes in their physical and social environment as a result of poorly designed enclosures, changes in food presentation, and changes to their social structure. It is well known that stress induces behavioural changes (Dierauf 1990; Waples & Gales 2002) that could be related to prolonged adrenal activity and long-term welfare problems, making these individuals more susceptible to health problems (Broom & Johnson 1993). Animals that cannot afford adaptive responses and are unable to maintain homeostasis will develop pathological problems that impact the immune and reproductive systems (Sapolsky *et al* 2000; Chrousos 1998; Reeder & Kramer 2005). Plasma cortisol has proven to be useful in order to understand more about how dolphins cope with changes in their environment (Thompson & Geraci 1986; St. Aubin 1996; Ortiz & Worthy 2000). By measuring serum cortisol Suzuki *et al* (1998, 2003) have described the diurnal and annual cortisol changes in dolphins and killer whales. The studies mentioned above involved venipuncture; a procedure unable to be performed too frequently due to the high risk of infection from microbial agents as well as the potential for tissue inflammatory responses. In order to investigate cortisol levels over long periods it is crucial to have alternative, non-invasive procedures that will allow repeated cortisol sampling. Steroid measurement in saliva has proven to be a non-invasive procedure of great value when repetitive sampling is necessary as its collection is potentially stress-free and it remains a very practicable procedure (Walker *et al*; Stahl & Dörner 1982). The steroid hormones such as cortisol may diffuse into the acinar cells of the secretory end-piece of the salivary gland by virtue of their well-known solubility in the lipids of the cell membranes (Vining & McGinley 1987). The fraction that diffuses throughout the cell membranes is the free one and, as biological activity of steroid hormones is a function of the free fraction, salivary measurement is a useful indicator of the active levels of the hormone. The facts mentioned above induced us to develop a procedure that allows the measurement of salivary cortisol in live dolphins; precluding the need for them to be tied, immobilised or anaethetised. For this purpose we have measured cortisol concentration in dolphins trained to allow saliva sampling. Salivary concentration of cortisol was compared with time-matched blood samples. In addition long-term serial saliva sampling was performed from dolphins in two aquaria. So far no studies on salivary cortisol in dolphins have been published.

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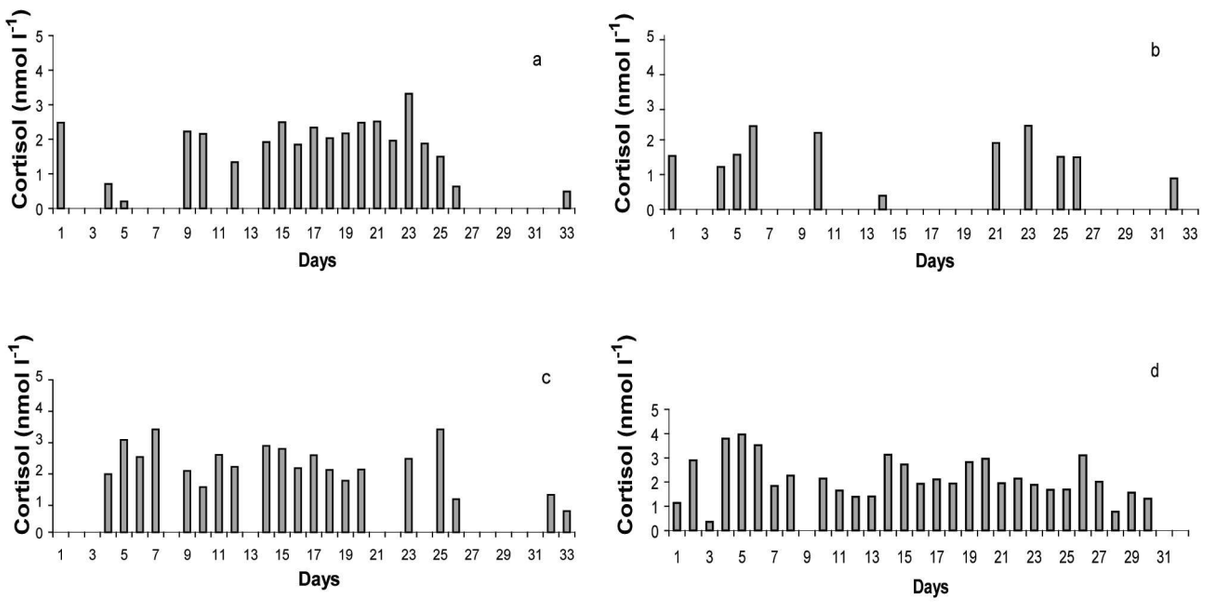
Figure 1



Serial saliva sampling in trained dolphins. Individual salivary cortisol profiles in female dolphins; 2A (Figure 1a) and 3A (Figure 1b) and male dolphins; 1A (Figure 1c) and 1B (Figure 1d). Dolphins were trained to voluntarily provide a saliva sample prior to the first meal of the day (0900–0930h). Each bar represents a single sample.

Materials and methods

Serial saliva sampling in trained dolphins

Four bottlenose dolphins held in two aquaria in Mexico City began training to give saliva samples in this study. The approximate age and time in captivity of those dolphins is shown in Table 1. The four dolphins were captured from the southern area of the Gulf of Mexico. Aquaria A and B have water capacities of 2,500 m^3 and 1,800 m^3, respectively. The quality of the water in both aquaria was similar as the same company administered them. The aquaria were opened to the public and both regularly hosted entertainment and assisted therapy sessions. Aquarium A has four show sessions a day, five days a week. The other two days were dedicated to dolphin therapy sessions. In aquarium B two daily show sessions were carried out during the whole week.

The dolphins participating in this study were trained until they learnt to emerge from the water and open their mouths voluntarily thereby assuring a non-invasive procedure with which to obtain the samples. Serial sampling was carried out before the first meal of the day which was always between 0900 and 0930h. Prior to saliva collection cotton swabs were washed with ether (which evaporated completely before use). To collect saliva, the swab was introduced up to the base of the tongue and placed in a sterile plastic tube. The swabs were then centrifuged at 3,000 rpm and the sample of saliva was stored at -20ºC until assayed. Due to the fact that it wasn't always possible to sample each dolphin every day, the total number of cortisol determinations was as follows: 20 for male 1A, 27 for male 1B, 19 for female 2A, and 11 for female 3A.

Serum and saliva time-matched samples

Serum and saliva time-matched samples were obtained for a total of seven dolphins from an aquarium in Nuevo Vallarta, Mexico. The approximate age and time in captivity of those dolphins is shown in Table 2. Four dolphins responded to training for saliva and blood sampling, while the other three were not trained and were handled during transportation to take both blood and saliva samples.

Blood was collected by venipuncture into vacuum tubes from the ventral aspect of the fluke, presented voluntarily and held in the appropriate position until sampling was completed as signaled by the trainer. In this group of dolphins (Table 2) saliva was also collected 3 to 4 minutes after blood collection, as described above. The samples from trained dolphins in the New Vallarta aquarium were always collected between 1000 and 1100h. The serum and saliva samples were stored at –20ºC until assayed.

Radioimmunoassay

Cortisol was directly assayed in serum and saliva using ^{125}I-Radioimmunoassay kits (Cort CT2, CIS Bio International, France). These samples were processed in duplicate.

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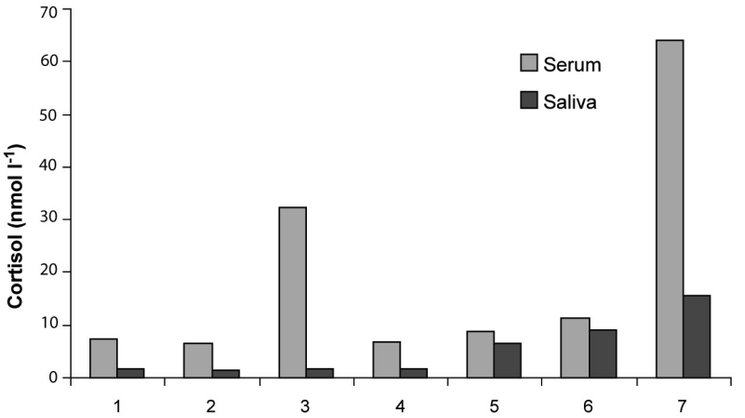
Figure 2

Dolphin serum saliva time-matched cortisol concentrations. Serum and saliva time-matched samples were obtained from 7 dolphins living in Puerto Vallarta aquarium. Samples were obtained between 1000 and 1100h. Saliva was obtained 3 – 4 min after blood collection.



Statistical analysis

In serial cortisol sampling descriptive statistics were performed. Pearson´s correlation test was used to relate blood cortisol and saliva cortisol values.

Results

Serial saliva cortisol measurements

The technique used in this study allowed frequent, non-invasive determination of a profile of cortisol in the saliva of the four dolphins sampled. Figure 1 (a,b,c,d) shows the individual values for each dolphin. The mean ± SD of serial sampling to determine cortisol concentrations in each dolphin was 1.8 ± 0.80 nm l^{-1} for Female 2A; 1.59 ± 0.62 nm l^{-1} for female 3A; 2.12 ± 0.70 for male 1A, and 2.14 ± 0.87 for male 1B. The highest levels coincided with events such as reproductive behaviour, exercise immediately prior to sampling, the presence of swimmers in the pool or mild sickness. Inter- and intra-assay coefficients of variation were 7.33% and 3.86%, respectively.

Time matched serum-saliva cortisol measurements

Data show that serum cortisol concentrations ranged from 6.59 to 64.46 nm l^{-1} in the seven dolphins sampled (19.70 ± 21.78, mean ± SD), while salivary cortisol concentrations ranged from 1.43 to 15.72 nm l^{-1} (5.43 ± 5.41, mean ± SD). Saliva values represented approximately 27% of blood values. The highest blood and saliva cortisol values were found in a young female captured a year before the study. On the other hand, dolphins 5 and 6, that were also under 5 years of age and recently captured, had similar values to those that had been captured 5 years ago. Figure 2 shows the matched serum-saliva cortisol concentration obtained in the 7 dolphins studied. The correlation value for serum and saliva cortisol concentrations was significant (0.73, n = 7, $P < 0.05$) indicating that a positive relationship was found between the serum and saliva time match values.

Discussion

This paper demonstrates, for the first time, that cortisol is secreted through the salivary glands in the dolphin.

Table 1 Approximate age and period of time in captivity of dolphins trained to provide serial saliva samples.

Dolphin	Approximate age (years)	Time in captivity (years)
Male 1A*	8	2.5
Male 2B*	15	9
Female 2A	20	7
Female 3A	15	10

*A and B indicate corresponding aquaria.

Table 2 Approximate age and period of time in captivity of the seven dolphins sampled for serum and saliva time-matched study.

Dolphins	Approximate age (years)	Time in captivity (years)
1 to 4	9-11	5*
5 to 7	4-5	1

* Dolphins trained to give samples.

Considering the fact that most dolphins sampled in this study were trained to open their mouth voluntarily, the hormone concentrations obtained from those dolphins could be considered as resting, non-stressed cortisol values. A similar assumption was made for the blood sampling by St. Aubin *et al* (1996) and Suzuki *et al* (1998, 2003), who considered that dolphins trained to voluntarily offer their tail flukes to allow blood collection, more likely reflect true resting values. The time-matched blood-saliva cortisol data obtained in the present study strongly suggest that the cortisol concentration in saliva is approximately 27% of the total plasma level. This percentage is close to that found in humans, with values provided ranging from 6.7 to 34.8% (Francis *et al* 1987), as well as squirrel monkeys (22%) (Tiefenbacher *et al* 2003). Numerous clinical studies have demonstrated that salivary cortisol concentrations are an accurate reflection of free plasma levels and are not affected

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by changes in corticotropin binding globulin (CBG) (Vining & McGinley 1987). This transportation protein varies throughout many physiological states, often complicating the interpretation of plasma cortisol levels. The collection of saliva for measuring cortisol greatly facilitates multiple sampling, allowing long-term serial sampling, and eliminating the problem of blood loss. Several research groups have proposed that salivary cortisol assay should become the method of choice to assess adrenal cortical function in humans (for a review see Vining & McGinley 1987). Captive dolphins can be trained; a fact that readily facilitates saliva sampling.

In summary, this study demonstrates, for the first time, that dolphins secrete measurable quantities of cortisol in their saliva. The technique described here allows frequent, non invasive determinations of cortisol to be performed in captive dolphins. Differences in cortisol profiles between dolphins sampled in this study could be the result of the period of time each individual had spent in captivity and hence a reflection of either habituation to being sampled or the lack of an adrenal response. The combination of this technique with clinical and behavioral observations could allow objective determination of the occurrence of stress-related medical problems in these marine mammals.

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References

Broom DM and Johnson KG 1993 *Stress and Animal Welfare*. Chapman & Hall: London, UK

Chrousos GP 1998 Stressors, stress, and neuroendocrine integration of the adaptive response. The 1997 Hans Selye Memorial Lecture. *Annual New York Academy of Science 851:* 311-35

Dierauf LA 1990 Stress in Marine Mammals: Marine mammal behavioral diagnosis In: Dierauf LA (ed) *CRC Handbook of Marine Mammals Medicine: Health, Disease and Rehabilitation* pp 295-301. CRC Press: Boca Raton, Florida, USA

Francis SJ, Walker RF, Riad-Fahmy D, Hughes D, Murphy JF and Gray OP 1987 Assessment of adrenocortical activity in term newborn infants using salivary cortisol determinations. *The Journal of Pediatrics 11:* 129-133

Ortiz RM and Worthy GAJ 2000 Effects of capture on adrenal steroid and vasopressin concentrations in free-ranging bottlenose dolphins (*Tursiops truncatus*). *Comparative Biochemistry and Physiology 125(2):* 317-324

Reeder D and Kramer KM 2005 Stress in free ranging mammals: integrating physiology, ecology, and natural history. *Journal of Mammology 86:* 225-235

Sapolsky RM, Romero LM and Munck A 2000 How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Review. 21:* 55-89

St. Aubin DJ 1996 Dolphin Thyroid and adrenal hormones: circulating levels in wild and semi-domesticated *Tursiops truncatus*, and influence of sex, age and season. *Marine Mammal Science 12:*1-13

Stahl F and Dörner G 1982 Responses of salivary cortisol levels to stress-situations. *Endocrinologie 80:* 158-162

Suzuki M, Tobayama T, Katsumata E, Yoshioka M, and Aida K 1998 Serum cortisol levels in captive killer whale and bottlenose dolphin. *Fisheries Science 64:* 643-647

Suzuki M, Uchida S, Keichi U, Tobayama T, Katsumata E, Yoshioka M and Aida K 2003 Diurnal and annual changes in serum cortisol concentration in Indo-Pacific bottlenose dolphins, *Tursiops aduncus* and killer whales, *Orcinus orca*. *General Comparative Endocrinology 132:* 427-433

Tiefenbacher S, Lee B, Meyer JS and Spealman RD 2003 Noninvasive technique for the repeated sampling of salivary free cortisol in awake, unrestrained squirrel monkeys. *American Journal of Primatology 60:* 69-75

Thomson CA and Geraci JR 1986 Cortisol, aldosterone, and leucocytes in the stress response of bottlenose dolphins, *Tursiops truncatus*. *Canadian Journal of Fisheries and Aquatic Science 43:* 1010-1016

Vining RF and McGinley RA 1987 The measurement of hormones in saliva: possibilities and pitfalls. *Journal of Steroid Biochemistry 27:* 81-94

Walker RF, Riad-Fahmy D and Read GF 1978 Adrenal status assessed by direct radioimmunoassay of cortisol in whole saliva or paratiroid saliva. *Clinical Chemistry 24:* 1460-1463

Waples KA and Gales NJ 2002 Evaluating and minimising social stress in the care of captive bottlenose dolphins *(Tursiops truncatus*). *Zoo Biology 21:* 5-26

https://doi.org/10.1017/S0962728600030682 Published online by Cambridge University Press