The characteristics of adrenal glands and its hormones in cetaceans

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SUMMARY: Cortisol is a member of the corticoid family and is known as a stress hormone. In cetaceans, cortisol is frequently measured as a stress indicator. However, inappropriate estimation of cortisol concentration frequently occurs due to a lack of fundamental knowledge of corticoids in cetaceans. We carried out several experiments to establish standard methods of cortisol measurement in cetaceans. 1) Cortisol biosynthesis was confirmed in Antarctic minke whales *Balaenoptera bonaerensis*. Corticoids including cortisol, progestins, adrenal androgens and many other intermediates were synthesized from ¹⁴C-pregnenolone. 2) Serum cortisol concentrations in 15 cetaceans species were measured. Average cortisol concentrations in each species showed a wide range from 2.9 ng/ml in killer whale *Orcinus orca* to 39.9 ng/ml in striped dolphin *Stenella coeruleoalba*. A negative correlation between cortisol and body length was confirmed. 3) Diurnal changes in cortisol concentrations were investigated in well-trained Indo-Pacific bottlenose dolphins *Tursiops aduncus* and in killer whales. Cortisol concentrations in both species exhibited episodic fluctuations with decreases in the evening and increases in the early morning. Annual cortisol changes in killer whales were also investigated, and pattern varied according to sex. 4) Changes in serum cortisol concentrations following transport into captivity in a male rough-toothed dolphin *Steno bredanensis* were investigated. Cortisol concentrations were high on the day of transport, and continued to decline until stabilizing about four months later. 5) Twenty-one-deoxycortisol concentrations increased with time after transport, and a significant negative correlation between cortisol and 21-deoxycortisol was demonstrated in a rough-toothed dolphin. This suggested that 21-deoxycortisol is useful as a negative indicator of stress.

KEY WORDS: cortisol, corticoids, cetacean, dolphin, whale, metabolism, 21-deoxycortisol

INTRODUCTION

The adrenal gland is a secretory organ existing in all vertebrates. The gland consists of an outer cortex and inner medulla. The cortex is responsible for the synthesis and secretion of steroids, while the medulla synthesizes and secretes catecholamines. Corticoid secretion is stimulated by adrenocorticotrophic hormone secreted from the pituitary under the control of corticotropin releasing hormone originating in the hypothalamus. The steroids can be grouped into three classes on the basis of their physiological effects: glucocorticoids, mineralocorticoids, and sex steroids.¹ Cortisol is a representative glucocorticoid and is used as an indicator of stress in individuals, because its secretion is responsive to stressful conditions in many types of animals. In terrestrial mammals, many studies on cortisol have been conducted, and a wide body of knowledge on cortisol, for example, metabolic pathways, fluctuating ranges, average concentrations,

and diurnal and annual variations, has been accumulated.

Studies on the adrenal gland in cetaceans have been reported since the eighteenth century.²⁻⁶ Early studies were mostly anatomical. Following the 1940's, biological studies using instrumentation that examined the specific density of various organs revealed that adrenal weight decreases as body weight increases.^{3,7-9} Following the 1960's, investigations on adrenal steroids commenced and various studies reported that the composition of steroid hormones in the adrenal glands of cetaceans was similar to that of terrestrial mammals,¹⁰ and that cortisol concentrations in the former tended to be lower than those in the latter. In the 1980's, cortisol concentrations began to be measured as a stress indicator in cetaceans.¹¹⁻¹⁸

Today, various controversies surround cetaceans, especially concerning whaling and the propriety of cetacean captivity in aquariums. However, almost of these arguments are based on not scientific, but moral

points of view. Measurement and evaluation of cortisol concentrations in cetaceans can provide useful and objective data for properly evaluating these arguments, but cortisol indices have not been available due to the absence of detailed knowledge on cortisol in cetaceans. It is now necessary to establish fundamental knowledge on cortisol and related steroids in order to develop useful scientific indicators of stress.

Metabolic pathway of adrenal steroids

Corticoid metabolism in the adrenal cortex of cetaceans *in vitro* has not been investigated thus far except for a study in stranded whales.¹⁹ Moreover, cortisol synthesis in the adrenal cortex has never been confirmed although cortisol has been detected in cetacean blood.

In order to examine corticoid metabolism in whales, adrenal glands were collected postmortem from eleven Antarctic minke whales in the Japanese research take of 1999/2000, and corticoid synthesis in the cortex was verified.

Aerobic incubations of cell free homogenate (CFH) of the cortex with ¹⁴C-labeled pregnenolone allowed the isolation of progesterone, 17-hydroxyprogesterone, cortisol, corticosterone, adrenal androgens, and many other intermediates. From the results of incubation of CFH with ¹⁴C-progesterone and that with ¹⁴C-17-hydroxyprogesterone, the pathway of adrenal steroids was confirmed and enzyme activities of 3 β -hydroxysteroid dehydrogenase/isomerase, P450_{17 α ,17 β} , P450_{c21}, and P450_{11 β} were revealed to be strong in Antarctic minke whales. This is the first study in cetaceans that demonstrated the biosynthesis of cortisol and 21-deoxycortisol *in vitro*.

Ranges and average concentrations of cortisol

Serum cortisol concentrations in 15 cetacean species (n=1,351) were measured by radioimmunoassay (RIA). Cortisol concentrations showed a wide range, from 2.9 $\pm$ 2.9ng/ml (mean $\pm$ SD) in killer whales to 39.9 $\pm$ 14.3ng/ml in the striped dolphin, and average concentrations were generally lower than previously reported values in cetaceans and terrestrial mammals. This is because blood samples obtained from well-trained captive individuals were included.

Significant negative correlation between cortisol concentration and body size was observed in the 15 species (p<0.05), and a stronger negative correlation was shown in 12 captive cetacean species (p<0.01). In addition, serum cortisol concentrations in the morning

and body weights showed the same negative correlation in normal adult individuals trained to undergo the husbandry blood sampling method at Kamogawa Sea World (p<0.05). This correlation indicates that cortisol plays a role in energy production in the body, because the amount of energy demand is in inverse proportion to the body size, although additional studies are needed to confirm this hypothesis.

Diurnal and annual variations in Cortisol concentrations

In cetaceans, diurnal changes in cortisol concentrations have been seldom investigated. Judd and Ridgway (1977) carried out through day sampling in bottlenose dolphins and reported that cortisol concentration decreases toward 18:00 and some peaks occurred at midnight.²⁰ However, it was unlikely that blood samples could be obtained without the influence of stress on their cortisol concentrations as these animals had been kept on a stretcher inside a container throughout the process of blood sampling.

We obtained blood samples from two captive killer whales and two Indo-Pacific bottlenose dolphins by the husbandry method at an interval of three hrs for twenty-four hrs and serum cortisol concentrations were measured by RIA. Cortisol levels in both of these species showed the same tendency to decrease at 18:00 and increase during the next morning. Especially in dolphins, cortisol concentrations were significantly lower at 18:00 and higher at 3:00. In the present study, it is suggested that cetaceans are diurnal animals because they show the same diel rhythms in serum cortisol concentrations as those of diurnal terrestrial mammals.

Annual changes in serum cortisol concentrations of three killer whales were also studied at an interval of two weeks for one year from 1996 to 1997. Blood sampling was carried out at approximately 9:00 by the husbandry method. Cortisol levels in both males showed seasonal variations with higher levels in winter and lower levels in summer. However, the female showed a cortisol cycle with a decline at a rate of once per 4.7 months. A few studies not on annual, but on seasonal variations in cortisol concentrations have been reported thus far in cetaceans.^{14,16} In this study, annual changes and difference according to sex were demonstrated for the first time.

Changes in cortisol concentrations after transport under captive conditions

Cortisol is known as a stress hormone; however, the

usefulness of cortisol concentration as a physiological indicator of stress has not been examined in cetaceans. In 1998, we investigated changes in serum cortisol concentrations for 18 months after transport to the Okinawa Expo Aquarium in a rough-toothed dolphin. All blood samplings were carried out during 9:00-10:00 except for the day of transport.

A high concentration was observed on the day of transport and levels fluctuated around 20ng/ml for two months; however, levels gradually declined these after and stabilized at approximately 10 ng/ml three to four months later. A similar tendency was observed in white whales and killer whales transported into Kamogawa Sea World. The decrease in cortisol concentrations after transport demonstrates that cortisol levels may serve as a useful stress indicator. These results also show the necessity of long-term investigations for evaluating acclimatization in cetaceans.

21-Deoxycortisol

Levels of electrolytes, white blood cells, thyroid hormones and aldosterone have been reported as sensitive indicators of stress in cetaceans. However, attention can now be focused on 21-deoxycortisol.

Twenty-one deoxycortisol is a precursor of cortisol synthesized in the adrenal cortex. It cannot be detected in the blood of terrestrial mammals except in human patients afflicted with congenital adrenal hyperplasia and Stein-Leventhal syndrome.²¹⁻²³ In our study, steroid hormones extracted from blood samples of white whales *Delphinapterus leucas*, Antarctic minke whales, bottlenose dolphins *Tursiops truncatus*, and killer whales were separated by high performance liquid chromatography, and 21-deoxycortisol concentrations in eluted fractions of the hormone were measured. Results showed that 21-deoxycortisol concentrations in several cetacean species were equivalent to corresponding cortisol concentrations. However, 21-deoxycortisol was not detected in the blood of a wild bottlenose dolphin caught by drive fishing; most likely, it was not converted from 17-hydroxyprogesterone, the precursor of 21-deoxycortisol. Moreover, 21-deoxycortisol concentrations gradually increased after transport into captive conditions in a rough-toothed dolphin, and it was negatively correlated to cortisol concentration ($p < 0.05$). These results indicate that 21-deoxycortisol is not secreted when an animal is under stressful conditions, but it begins to be secreted when the animal is unstressed. Further studies are planned to investigate this area.

In conclusion, a great deal of information on

corticoid and serum cortisol concentrations were obtained in our studies. The evaluation of cortisol concentrations as a stress indicator is expected to be useful, especially in captive cetacean species.

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REFERENCES

1. Brooks, RV. 1979. Biosynthesis and metabolism of adreno-cortical steroids. In VHT. James (ed) *The adrenal gland*: Raven Press, 67-92pp.
2. Hunter, J. 1787. Observations on the structure and oeconomy of whales. *Phil. Trans. Roy. Soc. London* 77: 371-450.
3. Quiring, DP. 1943. Weight data on five whales. *J. Mammal.* 24: 39-45.
4. Burn, JH, Langemann, H., and Parker, RHO. 1951. Noradrenaline in whale suprarenal medulla. *J. Physiol.* 113: 123-128.
5. Arvy, L. 1971. Endocrine glands and hormonal secretion in cetaceans. In G. Pilleri (ed) *Investigation on cetacea* vol. III (2), Berne, 229-300pp.
6. Shimplon, JG and Gardner, MB. 1972. Comparative microscopic anatomy of selected marine mammals. In Ridgway, SH (ed) *Mammals of the sea*, Springfield, IL. : 386-392pp.
7. Crile, G and Quiring, DP. 1940. A comparison of the energy-releasing organ of the white whale (*Delphinapterus leucas*) and the thoroughbred horse "Equipoise". *Growth* 4: 291-298.
8. Slijper, EJ. 1958. Organ weights and symmetry problems in porpoises and seals. *Arch. Neerl. Zool.* 13: 97-113.
9. Harrison, RJ. 1959 Endocrine organs: hypophysis, thyroid, and adrenal. In Andersen, HT (ed) *The biology of marine mammals*, Academic Press, 349-390pp.
10. Race, GJ and Wu, HM. 1961. Adrenal cortex functional zonation in the whale (*Physeter catadon*). *Endocrinology* 68: 156-158.
11. Medway, W, Geraci, JR, and Klein, LV. 1970. Hematologic response to administration of corticosteroid in the bottlenose-dolphin (*Tursiops truncatus*). *J. Am. Vet. Med. Assoc.* 157: 563-565.
12. Kjeld, M and Olafsson, I. 1987. Some biochemical parameters in blood and urine of fin whale (*Balaenoptera physalus*). *Isr. J. Vet. Med.* 43: 117-121.
13. Kjeld, M and Theodorsdottir, A. 1991. Some electrolytes, hormones, and other substances in the blood of fin whales of the coast of Iceland. *Natturfraedingurinn* 60: 147-154.
14. Orlov, MM, Mukhlya, AM, and Kulikov, NA. 1988. Hormonal indices in the bottlenosed dolphin *Tursiops truncatus* in the normal conditions and during experimental

- stress. *Zh. Evol. Biokhim. Fiziol.* **24**: 557-563.
15. Koopman, HN, Westgate, AJ, Read, AJ, and Gaskin, DE. 1995. Blood chemistry of wild harbor porpoises *Phocoena phocoena* (L.). *Mar. Mamm. Sci.* **11**: 123-135.
 16. St. Aubin, DJ and Geraci, JR. 1989. Adaptive changes in hematologic and plasma chemical constituents in captive beluga whales, *Delphinapterus leucas*. *Can. J. Fish. Aqua. Sci.* **46**: 796-803.
 17. St. Aubin, DJ and Geraci, JR. 1990. Adrenal responsive-ness to stimulation by adrenocorticotrophic hormone (ACTH) in captive beluga whales, *Delphinapterus leucas*. *Can. Bull. Fish. Aquat. Sci.* **224**: 149-157.
 18. St. Aubin, DJ, Ridgway, SH, and Wells, RS. 1996. Dolphin thyroid and adrenal hormones: Circulating levels in wild and semidomesticated *Tursiops truncatus*, and influence of sex, age, and season. *Mar. Mamm. Sci.* **12**: 1-13.
 19. Carballera, A, Brown, JW, Fishman, LM, Trujillo, D, and Odell, DK. 1987. The adrenal gland of stranded whale (*Kogia breviceps* and *Mesoplodon europaeus*): morphology, hormonal contents, and biosynthesis of corticoid. *Gen. Comp. Endocrinol.* **68**: 293-303.
 20. Judd, HL and Ridgway, SH. 1977. Twenty-four hour patterns of circulating androgens and cortisol in male dolphins. In Ridgway, SH. and Benirschke, K. (eds) *Breeding Dolphin: Present status, Suggestion for the future*, Natl. Tech. Info. Serv., 269-277pp.
 21. Franks, RC. 1974. Plasma 17-hydroxyprogesterone, 21-deoxycortisol and cortisol in congenital adrenal hyperplasia. *J. Clin. Endocrinol. Metab.* **39**: 1099-1102.
 22. Maschler, I, Salzberger, M, and Finkelstein, M. 1976. Ovarian enzymatic divergence in patients with ovary syndrome excreting urinary pregnanetriolone. *Acta Endocrinol.* **82**: 366-379.
 23. Travaglini, P. and Faglia, G. 1971. Pregnanetriolone excretion in Stein-Leventhal syndrome. *Acta Endocrinol.* **68**: 826-832.