

Short Communication

## Circadian variations in salivary chromogranin a concentrations during a 24-hour period in dogs

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The purpose of this study was to determine if salivary chromogranin a secretion in dogs exhibits a circadian rhythm. Saliva sampling was performed during three different sessions occurring in three nonconsecutive 24-h periods. Sixteen healthy adult beagle dogs (8 males and 8 females) were moved to a sampling room and housed individually in cages. Saliva samples were obtained every 4 h from 12:00 p.m. to 12:00 p.m. the following day. In the interest of habituation, saliva was obtained hourly from each dog 3 h before the experiment was started. Salivary chromogranin A concentrations were measured using an enzyme-linked immunosorbent assay. No circadian rhythm was detected for salivary chromogranin A secretion, and no differences in salivary chromogranin A concentrations measured every 4 h were demonstrated during the 24-h cycle in dogs.

**Keywords:** chromogranin A, circadian variations, dog, saliva

Chromogranin A (CgA) exists in chromaffin granules of the adrenal medulla and is co-released with epinephrine or norepinephrine [2,3]. Currently, CgA is known to be stored in secretory granules in wide ranging neurons and endocrine paraneurons, along with neurotransmitters or hormones [8,18]. It has been shown that CgA is stored in the acinar cells of the salivary glands in rats and horses [14] and that it is released by sympathetic nerve stimuli in saliva [9]. Human salivary CgA has also been shown to be produced by the submandibular gland and secreted into saliva [13]. Nakane *et al.* [12] reported that salivary CgA had promise as a sensitive index of psychosomatic stress.

Akiyoshi *et al.* [1] reported that canine plasma CgA immunologically cross-reacts with human CgA and that measurement of the canine plasma CgA concentration may

provide a useful index for evaluating the acute stress response. However, 24-h internal secretion patterns must be considered since the circadian rhythm might influence CgA concentrations. To our knowledge, there has been no report concerning the circadian rhythms of plasma and salivary CgA concentrations in dogs. The advantage of using saliva is that it is obtainable without the discomfort of blood sampling. If there is a circadian rhythm in canine salivary CgA secretion, then such a pattern should be considered in future research on dog stress. The purpose of this investigation was to determine if salivary CgA secretion in dogs has a circadian rhythm.

Sixteen healthy beagle dogs (8 male and 8 female; age:  $3.40 \pm 0.59$  years) were used. Dogs were housed in the sampling room for three days for adaptation. The lights were turned on from 8:00 a.m. to 8:00 p.m., and the room temperature was kept between 20 and 25°C throughout the experiment. Water was given *ad libitum*, and dry food was provided at 8:00 a.m. The dry food was provided after saliva sampling. This experiment was approved by the Ethics Committee on Animal Experiments at the School of Veterinary Medicine and Animal Sciences, Kitasato University.

Experimental saliva sampling was performed during three nonconsecutive 24-h periods. The sixteen dogs were moved to the sampling room and housed individually in cages. Salivary sampling was done at 4-h intervals from 12:00 a.m. and 12:00 p.m. of the following day. However, in order to habituate dogs to saliva sampling, we sampled their saliva hourly starting 3 h before the experiment began. At night, saliva sampling was performed using a dim light.

Saliva was collected by inserting a cotton ball tied with a cotton thread into the dog's oral cavity for 1 min. The cotton ball was then suspended by a thread in a 10-ml centrifuge tube so it did not reach the bottom and was immediately centrifuged at 3,000 g at 4°C for 15 min. After centrifugation, saliva samples were stored at -20°C until analysis was performed. Salivary CgA concentrations

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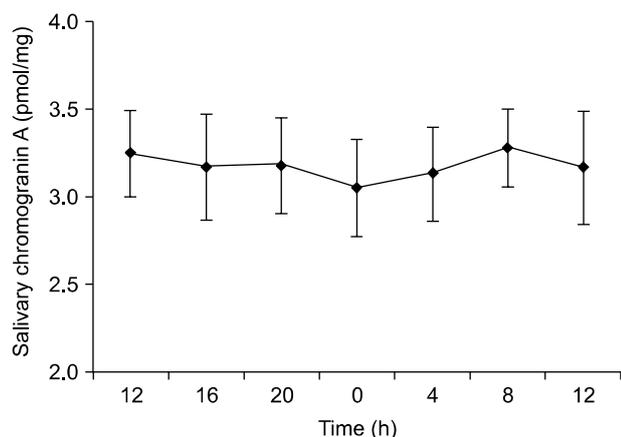
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were measured using a Human CgA enzyme-linked immunosorbent assay kit (Yanaihara Institute, Japan). All samples were analyzed in duplicate. Salivary CgA concentrations were measured as picomoles of CgA per milligram of protein.

Values are expressed as means  $\pm$  SD. Repeated measures ANOVA and Scheffe's test were used for analysis. Student's unpaired test was conducted to determine the mean values for male and female dogs at each collection time.  $p$  values  $< 0.05$  were considered statistically significant.

No circadian rhythm for salivary CgA was observed among the 16 dogs (Fig. 1). The highest concentration ( $3.28 \pm 0.22$  pmol/mg) of salivary CgA was observed at 8:00 a.m., and the lowest concentration ( $3.05 \pm 0.28$  pmol/mg) was observed at 0:00 a.m. However, no differences were noted in the salivary CgA levels measured every 4 h. There were no differences in salivary CgA concentrations between male and female dogs (Fig. 2). The highest and lowest concentrations of CgA measured in males ( $3.25 \pm 0.20$  and  $3.00 \pm 0.22$  pmol/mg) and in females ( $3.28 \pm 0.22$  and  $3.00 \pm 0.28$  pmol/mg) were recorded at 8:00 a.m. and 0:00 a.m.

Salivary sampling was performed 3 h before this experiment was started, because the salivary CgA concentration obtained during the first sampling was remarkably high. Moreover, the results were not influenced at the time of salivary sampling. When interpreting these results, we considered the possibility that a dog would undergo a stress reaction when the cotton ball was inserted into its oral cavity. As a result, the salivary CgA level peaked during the first sampling and then decreased 1 h later. Thereafter, the salivary CgA

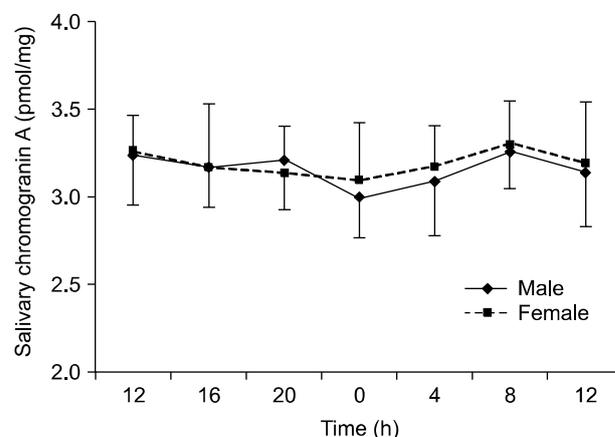


**Fig. 1.** Circadian variations in salivary CgA concentrations in 16 dogs during a 24-h period (mean  $\pm$  SD). Saliva sampling was performed on three nonconsecutive days. Sixteen healthy adult dogs (8 male and 8 female) were housed in individual cages. Salivary samples were taken at 4-h intervals between 12:00 a.m. and 12:00 p.m. the following day.

concentration remained low up through 3 h (data not shown). In this study, the dogs exhibited no circadian rhythm in salivary CgA secretion.

To our knowledge, there has been no report in the literature concerning the circadian rhythm of plasma and salivary CgA concentrations in dogs. In humans, plasma CgA secretion has been clearly shown to exhibit no circadian rhythm [4,16]. Recently, Den *et al.* [4] reported that the human salivary CgA concentration peaked upon awakening, quickly decreased to a nadir after 1 h, then remained at a low level throughout the day. In the present experiment, the highest concentration of salivary CgA was observed at 8:00 a.m., and the lowest level was observed at 0:00 a.m. the following day. This is in contrast to the lack of a circadian rhythm seen with human salivary CgA concentrations. Salivary cortisol concentrations, which serve as a useful stress index, are high in the morning and low in the afternoon in many mammals. However, Koyama *et al.* [11] reported no circadian rhythm in salivary cortisol concentrations in dogs and proposed that the circadian mechanism in dogs may be basically different from that seen in other species'. Moreover, a number of investigators have failed to detect a circadian rhythm in canine hormones [5-7,10,15,17].

Our study is the first known investigation of the salivary CgA circadian rhythm in dogs. In humans, salivary CgA secretion might be a sensitive index of psychosomatic stress [12]. Akiyoshi *et al.* [1] indicated that measurement of plasma CgA concentrations might provide a useful index for evaluating the acute stress response in dogs. Therefore, an understanding of the circadian rhythm associated with salivary CgA secretion in normal dogs is also important. Additional studies are warranted to better understand salivary CgA concentrations and their relationship to the canine stress response.



**Fig. 2.** Time course of salivary CgA concentrations in male and female dogs (mean  $\pm$  SD).

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