Salivary Secretory IgA Concentrations in Beagle Dogs

Aya KIKKAWA1*, Yoshiko UCHIDA1, Tetsuya NAKADE1 and Kiyoshi TAGUCHI1

1/School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai-Midorimachi, Ebetsu 069–8501, Japan

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ABSTRACT. The normal concentrations of salivary secretory IgA (sIgA) were examined, and the response of sIgA to acute stress was evaluated in dogs. Ten clinically healthy beagle dogs familiarized with the method of saliva sampling were used. During the non-stress period, saliva samples were collected between 0800 hr and 1700 hr at 1-hr intervals for 7 consecutive days and analyzed for sIgA concentration. After a 1-day control period, a noise stressor was presented for 15 min between 0845 hr and 0900 hr on 2 consecutive days. Saliva was collected at pre-stress, immediately after, 30 min after and 60 min after the stress. The average sIgA concentration over the 2-day period was compared with the control value. Environmental stimuli were restricted. During the non-stress period, significant variations were observed during the diurnal pattern, in which sIgA increased in the morning and then decreased; and the day-to-day variations were significant except at 0800 hr and 0900 hr. During the stress experiments, the sIgA concentration decreased significantly, immediately after and 30 min after the noise stress, and then increased to the same level as the control value by 60 min after the stress. When estimating the effectiveness of salivary sIgA as a marker of stress in dogs, the appropriate time for saliva sampling appears to be in the morning. Salivary sIgA was deemed potentially useful as a marker of stress in dogs.

KEY WORDS: canine, salivary sIgA, stress.

Salivary secretory immunoglobulin A (sIgA) has been shown to be an objective and sensitive marker of stress in humans [1–3, 5, 7, 11–13, 15–18]. Samples are easily obtained without causing undue distress to the subject. In dogs, however, little has been done to clarify the possible relationship between sIgA and stress. Reporting that salivary sIgA concentration in police dogs diminishes when the animals are engaged in training or encountering changes in their environment, Skandakumar et al. contended that salivary sIgA may provide a reliable marker of stress in dogs [14]. On the other hand, German et al. found wide variations in salivary sIgA in individual dogs, leading them to conclude that it is not feasible to use salivary sIgA concentration as a physical marker, such as an indicator of stress in dogs [6]. Based on this controversy in the literature, the question arises as to whether variations in salivary sIgA result from stress responses to the method of sampling or to environmental stimuli. We hypothesized that if stressors were eliminated from the experimental protocol, the salivary sIgA concentrations per dog would be more stable and would represent normal values.

Although human research has repeatedly acclaimed the use of salivary sIgA as a noninvasive marker of stress, particularly psychological acute stress [11–13, 15–18], veterinary work in this field is at a standstill. The current unavailability of canine sIgA reference values is an underlying drawback to objective assessment of the potential usefulness of salivary sIgA as a noninvasive marker of stress in dogs.

The objective of the present study was twofold: first, to examine the salivary sIgA concentrations in dogs; and second, to evaluate the response of salivary sIgA concentration to acute stress and compare with the values obtained from the non-stress period of the experiments. The points of departure from the study by German et al. [6] were that the dogs in the present work were thoroughly familiarized with the method of saliva sampling before the experiments began and, in the first part of the study, visual and audio stimuli were shut out in order to address the variations described by German et al. [6] namely, diurnal and day-to-day variations.

Thus, by first determining normal indices and then testing them against parameters obtained from the dogs under acute stress, this paper is expected to enable a reassessment of the potential usefulness of salivary sIgA as an objective marker of stress in dogs.

MATERIALS AND METHODS

Animals: Ten healthy beagle dogs (5 males and 5 females), 11 to 12 months old and weighing 9.5 ± 0.7 kg, were used. The dogs were housed individually in cages (60 × 42 × 50 cm) in a well ventilated laboratory, and the circadian light/dark cycle was maintained by lighting the room by overhead fluorescent lights from 0800 hr to 2000 hr. The doors and windows of the laboratory were closed and shielded to restrict visual and auditory environmental stimuli.

The animals were handled according to the Laboratory Animal Control Guidelines of Rakuno Gakuen University, which are based on the Guide for the Care and Use of Laboratory Animals of the U.S. National Institutes of Health. All the dogs remained healthy and their body weight was within normal range for the duration of the experiments.

Acclimatization: Daily, the dogs were familiarized with the environment, care, and sampling protocol for 10 days preliminary to the experiments.

Experimental design: Non-stress period: Saliva was col-
obtained from 673 (96.14%) of the samples. Two-way analysis of diurnal and day-to-day variations was performed with a quantitation kit (Dog IgA Quantitation kit, Bethyl, Texas). Results are expressed in ELISA units (EU), where the concentration of the reference serum (1 mg/ml) was defined as 100 EU/ml. Six different saliva samples were analyzed, with 20 repeats each, and the inter-assay variation was between 4.3% and 10.8% (mean 7.2%).

**Stress period:** After a 1-day control period, on 2 consecutive days (day-1 and day-2) a noise stressor in the form of an activated vacuum cleaner (75–78 dB) was used for 15 min between 0845 hr and 0900 hr near the cages in the laboratory. The behavioral responses of the dogs were noted, and saliva was sampled 4 times daily according to the procedure used in the non-stress period. Saliva was collected at pre-stress (0830 hr), immediately after the stress, 30 min after and 60 min after the stress. Drinking and feeding were prohibited during sampling to control their possible influence on the slgA concentration.

**Statistical analysis:** Diurnal variations (0800 hr to 1700 hr) and day-to-day variations (day 1 to day 7) were determined by 2-way repeated-measure ANOVA (P<0.05 was considered significant and P<0.01 highly significant). Dunnett’s post hoc test was used to compare slgA concentrations at 0800 hr with those at other times (P<0.05 was considered significant).

To evaluate the response of slgA concentration to the noise stressor, repeated t-test for each sampling time was used to compare the average slgA concentrations of day-1 and day-2 with control values. To estimate the reliability of slgA response to acute stress, the correlation between the degrees of variation, by subtracting the control value from the slgA concentration, on day-1 and those on day-2 was calculated by Pearson’s correlation (P<0.05 was considered significant and P<0.01 highly significant).

**RESULTS**

**slgA concentrations during the non-stress period:** Of 700 samples (10 dogs × 10 samples × 7 days), sufficient saliva for analysis of diurnal and day-to-day variations was obtained from 673 (96.14%) of the samples. Two-way repeated-measure ANOVA showed significant diurnal variations (df=9, F=13.928, P<0.001), day-to-day variation (df=6, F=3.615, P=0.005) and interaction between these variations (df=54, F=2.9, P<0.001). Because interaction was significant, we analyzed the diurnal and day-to-day variations further on each sampling.

Throughout the non-stress period, the diurnal slgA concentrations varied significantly for each day (F[9, 81]=2.16, 3.31, 7.25, 5.39, 23.56, 9.97 and 8.29 for each day, P<0.05), disclosing a pattern in which the slgA concentration was lowest at 0800 hr, increasing steadily through the morning, and decreasing from around noon (Fig. 1). The slgA concentration was significantly (P<0.05) lower at 0800 hr than concentrations between 1000 hr and 1700 hr, as shown by Dunnet’s post hoc test.

Over the 7 days of testing, day-to-day variation was negligible in the 0800 hr and 0900 hr slgA concentrations (F[6, 54]=0.34 and 0.65, P>0.1), the mean coefficient of variation being 25.1% (ranging from 18.8–34.1%) at 0800 hr and 27.5% (2.7–45.8%) at 0900 hr. After 1000 hr, slgA concentrations varied significantly from day to day (F[6, 54]=2.89, 4.09, 19.67, 2.35, 3.52, 2.40, 2.30, and 4.14 for each time, P<0.05). The variations were gradually increased in the morning, and great variation was maintained over the next 5 hr, as seen by the coefficient of variation: 39.2% (10.3–71.6%) at 1000 hr, 57.3% (27.5–102.6%) at 1100 hr, 65.6% (39.7–103.5%) at 1200 hr, 59.1% (27.3–75.9%) at 1300 hr, 63.1% (32.4–100.1%) at 1400 hr, 82.4% (31.9–120.9%) at 1500 hr, 54.1% (33.0–68.2%) at 1600 hr, and 69.5% (36.8–101.5%) at 1700 hr.

**slgA concentrations in response to stress:** Enough saliva was obtained for analysis of the 120 samples (control period: 10 dogs x 4 samples; post-stressor period: 10 dogs x 4 samples x 2 days). Pre-stressor slgA concentrations were the same as control values (df=1, F=0.188, P=0.678), but...
immediately after stress infliction, the concentrations dropped to less than half those of the controls, which was highly significant (df=1, F=22.683, P=0.001). At 30 min post-stressor, the concentrations remained significantly lower (df=1, F=9.344, P=0.014), although the difference had become somewhat smaller. At 60 min post-stressor, the concentrations had returned to near control levels (df=1, F=1.257, P=0.261) (Fig. 2).

Pearson’s correlation test, used to evaluate the degree of variation between day-1 and day-2 concentrations, showed a significant positive correlation in the sIgA concentrations immediately after stress infliction (n=10, r=0.766, P=0.010) and 30 min after post-stressor (n=10, r=0.642, P=0.045) (Fig. 3). These results attest to the reliability of the sIgA response to acute stress induced by the vacuum cleaner noise.

Behaviorally, all 10 dogs exhibited stressful responses to the noise stressor, such as placing the tail between their legs, trembling and crouching down.

DISCUSSION

This study has documented for the first time a consistent pattern in diurnal concentrations of salivary sIgA in dogs, i.e., low in the morning, peaking around noon and attenuating in the afternoon. In addition, all individual concentrations at 0800 hr and 0900 hr were found to remain stable from day to day, thus representing what may be potentially useful reference values in beagles. Third, on sudden infliction of acute stress in the dogs, the salivary sIgA concentrations fell dramatically, not returning to the newly found reference values until 60 min later. Together, these results are thought to show promise for using salivary sIgA as a marker of stress in dogs.

In contrast to our diurnal results, in humans, Huck- lebridge et al. [10] reported markedly high concentrations of sIgA at the time of awakening and a decline over the next 4 hr, although the last 6 hr of the day had low concentrations. These differences may be partially ascribed to species-related behavior. For the most part, people keep active after awakening, whereas dogs, tethered to their doghouse, are more active in the morning and early evening but sleeping intermittently for long periods during the daytime [9]. Likewise, all the dogs in this study, housed in individual cages, were observed to be active in the morning and early evening, although the behavioral aspects of the animals were not our primary focus and, therefore, not formally recorded. This leads us to speculate that, in dogs, sIgA concentrations may subside with activity and increase with inactivity.

If sIgA variation is related to behavior, then, foreseeably, pet dogs with their behavior controlled by the owner may show diurnal patterns of sIgA concentration that differ accordingly. In human studies, gender, age and social class have also been implicated in different sIgA concentrations [4]. Such factors were beyond the scope of the present canine experiments. Further study would be necessary, using larger experimental populations of a variety of breeds in their respective environments.

In contrast with the canine salivary sIgA concentrations demonstrated by German et al. [6], the reference values obtained from the present 0800 hr samples were stable. Moreover, although German et al. reported inconsistent diurnal concentrations, a consistent pattern was revealed in the present experiments. A plausible explanation for this discrepancy may lie in the differences in study protocol. Of major importance, in the present study the environmental stimuli were restricted and saliva sampling was the sole sampling done each day, whereas German et al. collected tear and saliva samples simultaneously [6]. The tear sampling, done by rolling around a cotton swab in the dog’s third eyelid and lower lid for 1 min each in both eyes, may have caused discomfort and, thus, acted as a stressor. The experimental environment is not clear in their study. The
results of our study support the hypothesis that if stressors are eliminated from the experimental environment and protocol, the salivary slgA concentrations per dog would be more stable than in investigations not taking such factors into consideration. The sample size may be a second factor contributing to the difference in our results. We collected saliva samples ten times daily for 7 days, whereas German et al. took a single sample at 0800 hr on each of 4 days [6], which may have been inadequate for full analysis and comparison.

In agreement with German et al. [6], however, day-to-day variation was observed in the saliva collected in our study at times other than 0800 hr and 0900 hr. Particularly around noon the concentrations varied widely, but the explanation is elusive and documented information about the regulation system of slgA in saliva is scarce. The study of Hucklebridge et al. suggest that many hormones and neurotransmitters influence slgA secretion [10]. Because these are known to be regulated by endogenous cues as well as influenced by environmental factors, the slgA regulation system is complex. In the complex regulation of slgA, behavior is one of the prospective factors suspected to have strong bearing on the noon-time variation in this study. All the dogs were active in the morning on each of the 7 days, their activity levels varying later in the day, especially around noon.

In addressing the seminal work by German et al., we also gave consideration to the use of beagles as a study model. Beagles were not used by German et al. because this breed was at that time presumed to be predisposed to IgA deficiency [8]. However, the beagle dogs in the present study were all bred in our laboratory, and none of the previous blood tests from any of the dogs had shown any IgA deficiency. Therefore, we believe that using these beagles for estimating the slgA concentrations was not problematic in any way. On the contrary, a controlled study such as this one was enhanced by unifying the breed, especially in terms of data analysis and interpretation of results.

All dogs in the present study reacted strongly to the noise stressor. For experimental purposes, the 75–78 dB vacuum cleaner activated for 15 min appeared to incite intense stress in dogs, particularly in those animals that had been restricted from noise stress before, and their slgA concentrations were decreased. In studies to date, intense stressors apparently caused downregulation of slgA, and not-so-intense stressors may have induced upregulation. In a number of reports describing salivary slgA response to acute stress in humans [1, 5, 7, 11–13, 15–18], some show that slgA concentrations were increased by negative hedonic music or mental recall [1, 5, 7, 11–13, 15–18]. In spite of a temporary surge in slgA concentration possibly resulting from SAM activation, the end result can be a decrease because the transport of IgA across the epithelial barrier cannot be maintained, thus depleting IgA.

In dogs, the present results find affinity with those described by Skandakumar et al., who demonstrated that in police dogs slgA decreases because of chronic stress, such as hard training [14]. The only authors so far reporting a relationship between canine slgA and stress, Skandakumar et al. collected saliva samples at 0830 hr and 0900 hr in the dogs’ regular environment, where all the dogs had been familiarized with the sampling procedures before experimental collection.

The results of this study underline the importance of taking into consideration the appropriate time and conditions for saliva sampling when estimating the effectiveness of salivary slgA as a marker of stress in dogs. These results suggest that the most reliable sampling time is in the morning. Salivary slgA is thought to be useful not only as a marker of stress in dogs but also as an indicator of certain pathological conditions and ethological concerns. In human medicine, low levels of slgA have pointed to susceptibility to respiratory disorders [7]. Conceivably, then, slgA may be useful as a marker of kennel cough. In addition, Skandakumar et al. showed that high adaptive ability in police dogs was associated with consistently high slgA levels during their training [14]. This gives rise to the view that slgA concentrations might also be of use in estimating the ability for other service dogs.

The reference values obtained in the present study show promise as guidelines in establishing salivary slgA reference values in dogs.

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