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Catestatin, vasostatin, cortisol, and visual analog scale scoring for stress assessment in healthy dogs

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ABSTRACT

The neuroendocrine glycoprotein chromogranin A is a useful biomarker for stress in humans. Chromogranin A epitopes catestatin and vasostatin can be measured in dogs using radioimmunoassays. The objective of this study was to evaluate catestatin and vasostatin as canine stress biomarkers in a clinical setting. Blood and saliva were collected from 33 healthy dogs that were familiar with sampling procedures and the animal hospital environment (control group) and 30 healthy dogs that were unacquainted (stress group). During sampling, stress behavior was scored by the same observer using visual analog scale (VAS). Plasma was analyzed for catestatin and vasostatin, serum for cortisol, and saliva for catestatin. Differences between groups were analyzed using two-sample *t*-tests and $P < 0.05$ was considered significant. Stress behavior VAS score in the control group was significantly lower than in the stress group during blood ($P = 0.002$) and saliva ($P = 0.0009$) sampling. Serum cortisol and saliva catestatin concentrations in the stress group were higher than the control group ($P = 0.003$ and $P < 0.0001$, respectively). Serum cortisol concentrations were correlated with those of saliva ($r = 0.34$, $P = 0.04$) and plasma catestatin ($r = 0.29$, $P = 0.03$). Plasma catestatin and vasostatin did not differ significantly between groups. In conclusion, concentrations of saliva catestatin, and serum cortisol, and stress behavior VAS scores were significantly higher in the stress group. The results indicate that saliva catestatin may be useful as a biomarker for acute psychological stress in dogs.

1. Introduction

Fear and psychological anxiety, which are often experienced by animals when brought to a veterinary clinical practice, induce a stress reaction. This phenomenon is commonly known as the “white coat effect” and has been shown to lead to a fight or flight reaction through activation of the sympatho-adrenal-medullary (SAM) axis and the hypothalamic-pituitary-adrenal (HPA) axis (Marino et al., 2011; Höglund et al., 2012; Tennant, 2013). Although stress is essential for coping with acute changes in the body's homeostasis, the stress response i.e. secretion of catecholamines, chromogranin A (CgA), cortisol, and changes in physiological and behavior parameters (Moberg and Mench, 2000; Hekman et al., 2014) particularly if prolonged, can be detrimental and contribute to disease development in animals as well as in humans (Roizen, 1988; Moberg and Mench, 2000; Sapolsky et al., 2000; Hekman et al., 2014). Stress can be evaluated using both subjective and objective parameters such as behavior and measurement of cortisol and catecholamines in body fluids (Nakane et al., 1998; Akiyoshi et al., 2005; Hekman et al., 2014). However, all currently available methods have shortcomings and new assessment methods are needed.

Chromogranin A (CgA) has shown promise as a biomarker for evaluating stress in humans and a few studies have been done in pigs and dogs (Nakane et al., 1998; Akiyoshi et al., 2005; Lee et al., 2006; Toda et al., 2007; Escribano et al., 2013; Srithunyarat et al., 2017b). The glycoprotein CgA belongs to the Granin family and is stored in chromaffin granules and coreleased with catecholamines and neuroendocrine hormones from the adrenal medulla and sympathetic nerve endings when SAM is activated (Blaschko et al., 1967; O'Connor and Bernstein, 1984). Chromogranin A has a longer half-life, is more stable and easier to handle than catecholamines, and could, in the absence of

Abbreviations: CgA, Chromogranin A; CST, Catestatin; HPA, Hypothalamic-pituitary-adrenal axis; RPM, revolutions per minute; SAM, Sympatho-adrenal-medullary axis; VAS, Visual analog scale; VS, Vasostatin

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neuroendocrine tumors and gastrointestinal disease, therefore be an alternative marker for evaluating the SAM response (Blaschko et al., 1967; Crout, 1968; Derbyshire and Smith, 1984; O'Connor et al., 1989; Escribano et al., 2014). That CgA can be actively secreted into saliva has been shown to occur in rats, horses, pigs, and humans (Kanno et al., 1999; Sato et al., 2002; Saruta et al., 2005). Because saliva sampling is a noninvasive method, it may be preferable for stress monitoring purposes as the sampling itself, in humans, is less likely to induce a stress response than sampling of other body fluids or blood (Vincent and Michell, 1992; Nakane et al., 1998; D'Amico et al., 2014). In dogs, the CgA epitopes catestatin (CST) and vasostatin (VS) can be measured using radioimmunoassay in both blood and saliva and have been shown to be unaffected by age, gender, breed, and time of day (Srithunyarat et al., 2017b). However, no studies comparing CST, VS, cortisol, and stress score using stress behavior visual analog scale (VAS) between healthy dogs under nonstressful and stressful condition have previously been presented.

The aim of this study was to evaluate the potential of CST and VS as canine psychological stress biomarkers in a clinical setting. This study investigated concentrations of CST, VS, and cortisol, and stress behavior VAS score in healthy dogs where one group was accustomed to the sampling procedures and environment and the other was unaccustomed.

2. Materials and methods

2.1. Study design and ethical approval

This study comprises data and samples collected during two separate earlier studies (Srithunyarat et al., 2016; Srithunyarat et al., 2017b). These studies were approved by the Uppsala Ethical Committee (C301/12) and Khon Kaen University (KKU) Ethical Legislation (AEKKU 26/2557). All owners were informed and gave their consent prior to participation of their dog. No sedative drugs were used during or prior to the sampling procedures and owners were present throughout the clinical parts of the investigation.

2.2. Dogs

All dogs were healthy, and aged between one to eight years old. The average (mean ± SD) age, body weight, and body condition scores are illustrated in Table 1.

2.2.1. Stress group

Thirty privately-owned healthy female dogs, of ten different breeds, Chihuahua, Thai Ridgeback, Thai Bangkaew, Pomeranian, Shih Tzu, Maltese, Siberian Husky, Labrador Retriever, Poodle, and Mixed Breed, admitted for elective ovariohysterectomy at KKU Veterinary Teaching Hospital, were included in the study. All sampling and assessments were made prior to premedication and surgery as previously described (Srithunyarat et al., 2016). All dogs were regarded as healthy based on history, a complete physical examination (including assessment of the mental status, general attitude, appetite, mucus membrane appearance, capillary refill time, rectal temperature, body weight, body condition score, hydration status, hair and skin condition, heart and respiratory rate and sounds by auscultation, abdominal organs, musculoskeletal system and lymph nodes by palpation, and mouth, ear, and eye examination), and blood screening tests (hematology including hematocrit, hemoglobin, red blood cell, white blood cell, neutrophil, lymphocyte, monocyte, eosinophil, basophil, and platelet counts and blood biochemistry including creatinine, alanine aminotransferase, and total protein and parasite blood smears for *Dirofilaria immitis, Babesia cania, Hepatozoon canis, Ehrlichia canis, Trypanosoma evansi,* and *Anaplasma platys*). Dogs were unaccustomed to the sampling procedures and the animal hospital environment. Samples were collected between 8:30 a.m. to 11:30 a.m. Food was withheld for at least 6 h prior to sampling.

Table 1
Age, body weight and body condition score in 33 control and 30 stress dogs.

Parameters	Control group (n = 33)	Stress group (n = 30)
Age (months)	47 ± 25	28 ± 26
Body weight (kg)	35.8 ± 10.1	11.6 ± 7.0
Body condition score (of 9)	5 ± 1	5 ± 1

Data presented as mean ± SD.
Dogs in the control group were familiar with sampling procedures and the animal hospital environment whereas dogs in the stress group were unfamiliar with these events.

2.2.2. Control group

Thirty-three privately-owned healthy dogs that routinely donate blood at the University Animal Hospital (UDS), Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden, were included as previously described (Srithunyarat et al., 2017b). Twenty-four were males and nine females, of fourteen different breeds (Boxer, Bernese Mountain, Collie, Dalmatian, Flat Coated Retriever, German Shepherd Dog, Golden Retriever, Great Dane, Greyhound, Labrador Retriever, Leonberger, Shorthaired Pointer, White Shepherd, and Mixed Breed). These dogs were included as the control group as they were well accustomed to the sampling procedures and the animal hospital surrounding, and considered to express little or no signs of stress. Sixteen of these dogs visited the animal hospital on several occasions for blood donation, and in these dogs only one randomly chosen sampling occasion was included in this study. All dogs were healthy based on a complete physical examination and blood screening (hematology including hematocrit, hemoglobin, red blood cell, white blood cell, neutrophil, lymphocyte, monocyte, eosinophil, basophil, and platelet counts, and biochemistry including creatinine, alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, and albumin), blood type (DEA 1.1), and the presence of vector borne diseases including *Anaplasma phagocytophilum, Anaplasma platys, Borrelia burgdorferi, Ehrlichia canis, Ehrlichia ewingii, and Dirofilaria immitis* (Snap 4DX test, IDEXX Laboratories, Maine, USA). Samples were collected between 8:00 a.m. to 1:30 p.m. Food had not been withheld prior to sampling.

2.3. Sampling protocol

Blood and saliva samples were collected prior to evaluation of stress behaviors in all dogs included in the study. The sampling and scoring procedures were performed using the same methods and techniques in both dog groups. All scoring procedures were performed by one person only. All samples were collected within an hour after arrival at the respective hospitals.

2.3.1. Blood and saliva collection

Blood was collected from the distal cephalic vein using butterfly needles (BD Vacutainer, Becton-Dickson, Plymouth, United Kingdom) into lithium heparin tubes and clot activator tubes (BD Vacutainer, Becton-Dickson, Plymouth, UK) and centrifuged at 3300 rpm for 5 min. The obtained heparinized plasma and serum samples were freeze stored in cryotubes (Low Temperature Freezer Vials, VWR, Stockholm, Sweden). For practical reasons, in order not to interfere with the clinical work conducted at the different animal hospitals, the order in which blood and saliva samples were collected was randomized with an interval between saliva and blood sampling of < 10 min. Blood samples from the control dogs were collected by two veterinary nurses and from stress group dogs by one veterinarian (TS).

Saliva samples were collected using a swab (SalivaBio, Salimetrics, PA, USA) placed in the oral cavity for 60–90 s by the same veterinarian (TS) for all dogs. The swabs were centrifuged at 3000 rpm for 15 min and the saliva deposited was freeze stored.