Assessment of peripheral serotonin, cortisol and dehydroepiandrosterone in aggressive dogs

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Abstract

Canine aggression directed towards people is the most frequent reason for referral to behaviour practices. In order to provide new and improved diagnostic and therapeutic approaches for this problem, it is necessary to make an in-depth investigation of the biological basis of aggression in this species. The serotonergic system and the hypothalamic-pituitary-adrenal (HPA) axis are believed to play an important role in controlling aggression. The aim of the present study was to investigate both systems in aggressive (n=80) and control non-aggressive (n=19) dogs through the assessment of peripheral parameters, namely serum serotonin (5-HT), and plasma cortisol and dehydroepiandrosterone (DHEA). Moreover, the effect of the category of aggression and gender was investigated. Aggressive dogs showed significantly lower serum concentrations of 5-HT than non-aggressive dogs (278.5 vs. 387.4 ng/ml, P <0.01). The lowest 5-HT concentrations were found in the group of dogs showing defensive forms of aggression. Aggressive animals showed significantly higher plasma concentrations of cortisol than non-aggressive dogs (21.4 vs. 10.6 ng/ml, P =0.05). Finally, males as a whole showed significantly higher plasma concentrations of DHEA and DHEA/cortisol ratio values than did females (DHEA: 90.9 vs. 29.8 ng/ml, P <0.05; ratio: 9.5 vs. 3.8, P <0.01). The present results suggest that aggressive dogs might differ from non-aggressive dogs in the activities of the serotonergic system and the HPA axis.

Keywords: dog, aggression, stress, serotonin, cortisol, dehydroepiandrosterone

1. Introduction

Canine aggression, particularly when directed towards humans, is the most frequent behaviour problem presented at referral behaviour practices (Bamberger and Houpt, 2006; Fatjó et al., 2007). Moreover, it represents a problem concerning both public health and animal welfare issues since a high number of people are bitten by dogs every year (Overall and Love, 2001; Palacio et al., 2005) and a significant proportion of these animals end up being euthanized or abandoned (Hunthausen, 1997; Mikkelsen and Lund, 2000). Creating new tools for diagnosis and treatment of aggression in dogs, as well as improving existing therapeutic approaches, represents a major challenge for animal behaviour medicine today.

The understanding of the biological mechanism of canine aggression remains fragmentary. Serotonin and steroid hormones have shown to be critically involved in the control of this behaviour in several species.

Several studies in human as well as nonhuman primates show an inverse relationship between the concentration of the main 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) in cerebrospinal fluid (CSF) and aggression and impulsivity (Howell et al., 2007; Mehlman et al., 1994; Stanley et al., 2000). This finding has also been reported in canine species by one study which found that dominant aggressive dogs, especially those that did not display warning signals prior to biting (i.e. impulsive aggression), showed lower concentrations of CSF 5-HIAA than a group of non-aggressive dogs (Reisner et al., 1996). Others did not find this association (Lentz, 2000; Mertens, cited by Overall, 2005).

It is well known that blood 5-HT does not cross the hematoencephalic barrier. However, a correlation between blood and CSF serotonergic parameters has been found in humans (Sarrias et al., 1990). In addition, blood 5-HT content has been shown to be altered in some psychopathologies (Kovacic et al., 2008; Muck-Seler et al., 2004) and to be affected by drugs that act upon the central serotonergic system (Castrogiovanni et al., 2003; Fisar et al., 2008). In dogs, it has been recently reported that serum concentrations of 5-HT were lower in a group of aggressive animals compared with a control group (Çakiroglu et al., 2007).

Glucocorticoids also play an important role in aggression. Findings are contradictory and both high and low cortisol concentrations have been related to abnormal forms of aggression in humans (Haller et al., 2005). In animals, research on stress and aggression often focuses on issues related to social status. Among canids, in particular, higher faecal cortisol concentrations have been detected in dominant individuals (Creel et al., 1997; Sands and Creel, 2004). Several behaviourists highlight that a great proportion of privately-owned dogs displaying aggressive behaviour also show signs of stress and anxiety (Bamberger and Houpt, 2006; Reisner et al., 2007).

Along with glucocorticoids, adrenals also produce dehydroepiandrosterone (DHEA) and its sulfate derivative DHEAS, two neuro-active steroid hormones with antiglucorticoid properties affecting the brain (Maninger et al., 2009). They are also produced in the brain (neurosteroids) (Baulieu and Robel, 1998) and a correlation has been found between CSF and circulating levels (Guazzo et al., 1996). The production of neurosteroids may be a mechanism to counteract the negative effects of stress and return organims to homeostasis (Engel and Grant, 2001). Several recent studies have looked at DHEA and aggression (see review by Soma et al., 2008) but to the authors' best knowledge, there are not scientific evidences of the role of DHEA in canine aggression or stress.

A previous work by the authors showed the suitability of serum samples for the determination of 5-HT in aggressive and non-aggressive dogs (León, 2006; León et al., 2008). The aim of the present study was to assess the activities of the serotonergic system and the hypothalamic-pituitary-adrenal (HPA) axis in canine aggression directed towards humans. To this end, the concentrations of serum 5-HT and plasma cortisol and DHEA were analyzed in a group of aggressive and non-aggressive dogs. The influence of the category of aggression and the gender of the animals was moreover addressed.

2. Material and methods

2.1. Aggressive animals

A multicentric study was designed where two Spanish veterinary teaching hospitals (Universidad de Zaragoza and Cardenal Herrera-CEU, Valencia) contributed to the collection of cases from April 2004 to June 2008. Dogs included in the present study were referred to the Companion Animal Behaviour Services within the respective hospitals owing to problems of aggression towards people. Dogs showing play-related and predatory aggression directed to people were excluded. Displaying any other type of behavioural problem did not constitute an exclusion criterion.

In total, 80 dogs (52 males and 28 females) were included in the aggressive group. The mean age was 4.9 years old (ranging from 3 months to 14 years). The group consisted of dogs of 23 different breeds and their crosses and 10 small-medium mongrels.

Diagnosis of aggression was carried out by means of a detailed standard questionnaire on the dogs' behaviour and daily routine. Clinical classification of aggression was established in accordance with three main diagnostic criteria: target, context and dog's communicative signals (based on Fatjó et al., 2007). Three main pre-established diagnostic categories were then considered:

(1) SCA: Social Conflict-related Aggression directed towards family members. This might occur during status-related interactions and competitive or conflict situations. The dog might show defensive and/or offensive signals.

(2) DA: Defensive Aggression towards unfamiliar people. This might occur when approaching or manipulating the dog. The dog might show defensive signals.

(3) OA: Offensive Aggression towards unfamiliar people. This might occur when approaching or manipulating the dog. The dog might show offensive signals.

If a diagnosis did not fit into any of the previous categories, then it was labeled as (4) “other forms”, including aggression problems related to medical causes and/or pain/irritability conditions. Finally, if a dog showed more than one form of aggression, the one related to the reason for consultation was considered the main diagnostic category and the remaining as secondary diagnostic categories.

In order to detect any underlying causative or contributory medical condition to the aggression problem, all dogs were screened through physical examination, complete blood count, serum biochemistry and thyroid hormone measurement at the time of admission.

2.2. Control animals

The control group was made up of 19 dogs (8 males and 11 females) of 9 different breeds. The mean age was 4.3 years old (ranging from 11 months to 9.3 years). They were selected from a random sample of dogs from the hospital’s database (Universidad de Zaragoza) after behavioural and physical examination. The selected animals were healthy and lacked any history of aggression towards people and/or other dogs.

2.3. Sample collection and biochemical analyses

Blood samples (6 ml) were drawn from the jugular or cephalic vein into EDTA and anticoagulant-free tubes and centrifuged at 4500x*g* at 4ºC for 10 min. Aliquots of plasma and serum were frozen and stored at -30ºC and –80ºC, respectively. A part of serum aliquots was set aside for clinical analysis before freezing.

Serum 5-HT was measured in duplicate with a commercial EIA technique (Serotonin-ELISA, DLD Diagnostika GMBH, Hamburg, Germany). The intra and interassay coefficients of variations were 3.9-5.4% and 6%, respectively. Concentrations were expressed in ng/ml.

Plasma cortisol and DHEA were determined in duplicate using two home EIA techniques (Chacón, 2004). In the cortisol EIA validation test, the intra and interassay coefficients of variation were 3.5-6% and 3.9-9.9%, respectively. Regarding DHEA, the intra and interassay coefficients of variation were 7.4-8.8% and 8.3-9.05%, respectively. Concentrations were expressed in ng/ml. The DHEA/cortisol ratio was calculated.

2.4. Statistical analysis

Serotonin, cortisol, DHEA and DHEA/cortisol ratio were defined as dependent variables. A multifactorial multivariate analysis of variance was carried out to assess the effect of the factors “aggression” and “gender” on the concentrations of all biochemical parameters. In addition, a unifactorial multivariate analysis of variance was carried out to assess the effect of the factor “category of aggression”. Finally, correlations between all parameters in both groups of study were analyzed using the Pearson test.

Calculations were carried out using the statistical program SPSS 14.0. for Windows (SPSS, Inc, Chicago, USA). We considered that $P \leq 0.05$ denoted statistical significance.

3. Results

3.1. Description of aggression cases

The measurement of thyroid hormones suggested hypothyroidism in one dog (TSH 5.27 ng/ml and total T4 0.88 μg/dl; reference range: TSH 0.30-4.40 ng/ml and total T4 1.10-3.60 μg /dl) which was included in the diagnostic category SCA. Two dogs showed episodes of aggression related to epileptic seizures which were labelled as “other forms” of aggression (one as the main diagnosis and the other as a secondary diagnostic category). Animals showing pain and/or irritability-motivated aggression made up the rest of the individuals within the category “other forms”. In the rest of the animals, no detectable physical alterations contributing to aggression were found as a result of the tests.

SCA was the main diagnostic category in 71.25% of all aggressive dogs. Secondary diagnostic categories were detected in 35% of the dogs (Table 1). In addition, 63.6% of the dogs also displayed aggression directed towards other dogs. Finally, other concomitant behavioural problems were detected in 77.5% of the animals. The frequency of these problems was as follows: noise phobias (thunderstorms, fireworks, pitch noises, etc.) (41%); anxiety related problems (anxiety separation and generalized anxiety) (14.7%); inappropriate urination and/or defecation (11.6%), social fear (towards people or other dogs) (9.5%), compulsive disorders (9.5%), overactivity (6.3%), excessive attention-seeking behaviours (5.3%) and others (2.1%).

3.2. Analysis of biochemical parameters

The multifactorial multivariate analysis of variance showed a significant effect of the factors “aggression” ($P < 0.01$) and “gender” ($P < 0.05$) on the studied parameters. A non-significant interaction was detected between both factors. Mean concentrations of all biochemical parameters are shown in Table 2. Aggressive dogs showed significantly lower serum concentrations of 5-HT and higher plasma concentrations of cortisol than control dogs. Males showed significantly higher plasma concentrations of DHEA and DHEA/cortisol ratio mean values than females. No gender differences were detected for the rest of parameters.

The unifactorial multivariate analysis of variance showed a significant effect of the factor “category of aggression” ($P < 0.05$). Mean concentrations of all biochemical parameters for each category of aggression are depicted in Table 3.

DHEA was positively correlated with the DHEA/cortisol ratio both in the control (0.692; P <0.01) and in the aggressive group (0.448; P <0.01). Only in the aggressive group cortisol was negatively correlated with the ratio (-0.364; P <0.01) and positively correlated with DHEA (0.298; P <0.01).

4. Discussion

An important step in diagnosing behaviour problems is to rule out underlying organic causes. In the present study, medical examination and behavioural history revealed three causative or contributory types of medical conditions related to the aggression problem, namely hypothyroidism (one dog), epileptic seizures (two dogs), and pain and/or irritability (four dogs). Canine hypothyroidism may increase the likelihood of aggression by reducing the threshold for this behaviour, rather than be the direct cause (Fatjó et al., 2002). No strong support for a causative relationship exists, but hypothyroidism has been found to affect the turnover of 5-HT (Bauer et al., 2002). The relationship between epileptic seizures, 5-HT and aggression also remains unclear (Keele, 2005) but interestingly, an activation of the anterior thalamic nuclei seems to occur during aggressive motivation in rats, an area traditionally linked to seizure genesis (Ferris et al., 2008). Finally, it is suggested that a reduced serotonergic activity produces a generalized state of hyperirritability, lowering the threshold at which an organism responds to provocative stimuli (Berman et al., 1997).

Regarding the rest of categories, SCA accounted for most of the human-directed aggression diagnoses, followed by DA and OA. In spite of the varying terminology found in the literature, aggression directed towards owners, is reported to be the most common form of aggression directed towards humans by several animal behaviourist (Bamberger and Houpt, 2006; Fatjó et al., 2007; Landsberg et al., 1991). It is interesting to note that in more than one third of the cases, several forms of aggression towards people were detected simultaneously. Moreover, a large percentage of dogs (65%) also showed different forms of intraspecific aggression. In this regard, a recent study carried out in the Companion Animal Behaviour Service of the Universidad Autónoma de Barcelona found a significant association between defensive aggression towards people and defensive aggression towards dogs as well as between offensive aggression towards people and intrasexual aggression, suggesting a shared basic motivation mechanism for, respectively, defensive and offensive aggressive behaviour (Fatjó et al., 2007).

Serum concentrations of 5-HT in the control group (387.4 ng/ml) were similar to previously published data from whole blood in canine species (Chen et al., 1993; Ferrara et al., 1987; LaRosa et al., 1989). The aggressive group as a whole was characterized by significantly lower serum concentrations of 5-HT (278.5 ng/ml). This finding aligns with previous studies that find an inverse relationship between the concentration of 5-HIAA in CSF and aggression in several species, including dogs (Howell et al., 2007; Mehlman et al., 1994; Reisner et al., 1996; Stanley et al., 2000). With regards to the determination of peripheral 5-HT, a recent study also reported lower serum concentrations of 5-HT in a group of 33 dogs displaying aggression towards people and/or other dogs (12 ng/ml), as compared with 18 normal dogs (32.5 ng/ml) (Çakiroglu et al., 2007). Despite the same finding, it is worth mentioning that 5-HT concentrations in this study were very low in comparison with the present results, which may be explained in terms of methodological differences. Finally, the present results also support a preliminary study by the authors, where lower concentrations of 5-HT were simultaneously found in plasma, serum and platelets of 28 dogs that were aggressive towards people and/or other dogs compared with 10 non-aggressive dogs (León, 2006; León et al., 2008).

When considering the different categories of aggression, only dogs within the category SCA (277.7 ng/ml) and DA (235.8 ng/ml) showed significantly different serum concentrations of 5-HT to those in the control group. Since the lowest concentrations were detected in animals showing defensive forms of aggression, it could be argued that it is fear motivation in particular –rather than aggression motivation in general– that is linked with a low serotonergic activity. A previous study by DeNapoli et al. (2000) did not detect differences in plasma 5-HT between dogs showing dominance (mainly equivalent to SCA diagnosis) and territorial aggression (mainly equivalent to OA diagnosis). However, this study did not include a comparison with a group of non-aggressive dogs.

It has been consistently reported by animal behaviourists that male dogs are more frequently referred due to aggression problems than do females (APBC, 2005; Bamberger and Houpt, 2006; Fatjó et al., 2007). In fact, 65% of the total dogs recruited for the present study were males. Male and female dogs, however, did not significantly differ in serum concentrations of 5-HT. Despite the limited literature focusing on gender differences in the serotonergic system, sexual dimorphisms have been reported in the human brain. Several Positron Emission Tomography (PET) studies have shown that healthy women have higher 5-HT1A receptor and lower 5-HT transporter binding potentials (Jovanovic et al., 2008; Parsey et al., 2002) as well as lower rates of 5-HT synthesis (Nishizawa et al., 1997; Sakai et al., 2006) than healthy men. A recent CSF study in healthy volunteers, however, showed no differences in 5-HIAA concentrations between males and females (Nilsson et al., 2007). Serotonergic status in the blood of healthy humans was addressed in one study and differences between sexes were detected including plasma 5-HT and whole blood 5-HT (both higher in women), and plasma 5-HIAA (higher in men) (Ortiz et al., 1998). In spite of all of these findings, discussion of the current results remains difficult since none of the studies cited above specifically focus on sex differences in central or peripheral serotonergic measures of aggressive individuals. More studies with this aim, and also considering the role of sexual hormones, should be carried out in order to clarify this issue.

Plasma concentrations of cortisol in the control group (10.6 ng/ml) were within the normal range for canine species (Chacón, 2004). The aggressive group showed significantly higher plasma concentrations of cortisol (21.4 ng/ml). High cortisol concentrations have been associated with affective (hostile-reactive) aggression in different human subpopulations (serum, Soderstrom et al., 2004; saliva, van Bokhoven et al., 2005) as opposed to non-affective (instrumental-proactive) aggression, which is characterized by chronic cortisol deficiency (urine, Virkkunen, 1985; saliva, McBurnett et al., 2000). Affective aggression, either offensive or defensive, is characterized by an intense autonomic activation (Haller et al., 2005; Nelson and Trainor, 2007). It has been reported that many dogs that show aggression towards household members show ambivalent body language before an attack, which is believed to be an indicative of high arousal (Luescher and Reisner, 2008). Finally, it is interesting to note that a great proportion of the aggressive dogs also displayed concomitant behaviour problems, most of them related with fear/phobias and anxiety. This may also be related with a hyperactivity of the HPA axis in aggressive dogs.

Plasma concentrations of cortisol were significantly higher in the SCA group (23.2 ng/ml). Traditionally, canine aggression towards the owners has been related to a hierarchical conflict, thus receiving the name of "dominance aggression", where the dog responds aggressively in contexts related with competition for a resource (food, toy, resting place, etc.) and physical manipulation or punishment by the owner (Borchelt and Voith, 1996). Higher concentrations of faecal cortisol have been detected in dominant individuals in African wild dogs (Creel et al., 1997) and wolves, but this finding was not associated with high rates of aggression or agonistic interaction in the latter (Sands and Creel, 2004). Most probably higher plasma concentrations of cortisol found in aggressive dogs in the present study were related to a stress status rather than to a dominance status.

Males as a whole (both in the aggressive and control groups) showed significantly higher plasma concentrations of DHEA and values of DHEA/cortisol ratio (90.9 ng/ml and 9.5, respectively) than did females (29.8 ng/ml and 3.8, respectively). These results may fit with the observed fact that, in humans, women suffer more stress-related disorders such as anxiety and depression than men (Solomon et al., 2009). The mechanism underlying gender differences in DHEA(S) levels, however, is not known; some studies suggest sex steroids may be involved. Thus, testosterone seems to have a stimulatory effect and estradiol an inhibitory effect on adrenal androgen levels, consistent with higher levels in men than in woman (Laughlin and Barrett-Connor, 2000).

One possible limitation to the present work is the use of clinical data to classify aggression which can hinder accurate categorization of the basic aggressive motivation at the neurobiological level. Difficulties for clinical categorization have been acknowledged since it relies heavily on records of the context and the dog's signals during aggressive episodes, which are ultimately based on the owner's report. This is particularly true for cases in which ambivalent signals are observed or alternating offensive and defensive signals are shown by the animal. To counteract this inherent limitation, the authors complemented the diagnosis with data from the clinical history (mainly the origin and evolution of the problem). Another limitation is related to the unbalanced distribution of animals within the different categories of aggression. A larger number of individuals within the categories OA and "other forms" would allow a better assessment of the role of gender in the different groups of aggressive animals.

5. Conclusions

The present results suggest that dogs showing a problem of aggression towards people may differ from non-aggressive dogs in the activities of the serotonergic system and the HPA axis. The aggressive group, particularly those showing defensive forms of aggression towards unfamiliar people, was characterized by lower serum concentrations of 5-HT. In addition, aggressive animals showed higher plasma concentrations of cortisol. Regardless of the group, males showed higher plasma concentrations of DHEA and DHEA/cortisol ratios than females.

The determination of serum 5-HT may have important clinical applications in the future. For example, it could be used for deciding which animals might benefit from a given pharmacological treatment as well as for monitoring the response. The determination of plasma cortisol, and probably better, the determination of the DHEA/cortisol ratio, may be used for objectively assessing stress in aggressive animals. Regarding DHEA results, more studies should be performed in order to further explore the mechanisms underlying gender differences, as well as the role of this neuro-active steroid in canine aggression and stress.

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Table captions

Table 1. Number of dogs (percentage) displaying aggression within the different categories of aggression.

Table 2. Mean (standard error) concentrations of biochemical parameters according to the group and the gender of the animals.

Table 3. Mean (standard error) concentrations of biochemical parameters for each category of aggression and comparison with those in the control group.