RESEARCH

Assessment of serotonin in serum, plasma, and platelets of aggressive dogs

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Abstract Canine aggression is the most common reason for the referral of dogs to behavior practices. In addition, dog bites represent an important problem for public health and animal welfare. The serotonergic system is believed to play an important role in modulating aggression. The aim of the present study was (1) to assess the suitability of different types of blood samples for measuring circulating serotonin in canine clinical studies, and (2) to investigate the relationship between the serotonergic system and canine aggression. The assessment of serotonin was simultaneously carried out in serum, plasma, and platelets of 28 aggressive and 10 nonaggressive dogs with an enzyme immunoassay technique. The mean serotonin concentration in aggressive dogs was significantly lower than in nonaggressive dogs in all the assayed samples. These findings suggest an inverse relationship between the activity of the serotonergic system and canine aggression. Considering the simplicity of the methodology, the authors propose sampling serum as the most suitable method for measuring circulating serotonin in dogs.

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Introduction

Canine aggression is the most common reason for the referral of dogs to animal behaviorists (Bamberger and Houpt, 2006; Fatjó et al., 2007). Dog bites represent an important problem for public health (Overall and Love, 2001; Palacio et al., 2005). They also pose a problem in the field of animal welfare, as a significant proportion of aggressive dogs is euthanized or abandoned (Hunthausen, 1997; Mikkelsen and Lund, 2000). If these problems are to be prevented and effectively managed, it is necessary to deepen the understanding of the biological basis of canine aggression.

Serotonergic neurotransmission is believed to play a critical role in the control of aggression in several species (Ferrari et al., 2005). Low concentrations of the main serotonin (5-HT) metabolite 5-hydroxyindoleacetic acid in cerebrospinal fluid (CSF) have been associated with low thresholds for aggression or impulsivity in human and non-human primates (Mehlman et al., 1994; Stanley et al., 2000; Soderstrom et al., 2001; Howell et al., 2007) as well as in dogs (Reisner et al., 1996).

Technical and ethical restrictions linked to the study of the serotonergic system in psychiatric disorders through CSF and neurohistochemical studies have led scientists to search for more easily accessible biological samples. The
platelet where blood 5-HT is mainly stored (Kema et al., 2000) has been proposed as a peripheral marker of the serotonergic neuron (Plein and Berk, 2001). Although blood 5-HT does not cross the hematoencephalic barrier, a correlation between blood and CSF serotonergic parameters has been found in humans (Sarrias et al., 1990). In the realm of human aggression, the measurement of blood 5-HT has offered inconclusive results, as both low (Goveas et al., 2004) and high (Golubchik et al., 2009) concentrations have been found to be linked to aggressive behavior in different populations. In canine species, 2 recent studies have reported low serum serotonin concentrations in aggressive individuals (Çakiroglu et al., 2007; Rosado et al., 2010a).

The aim of the present study was 2-fold. First, to assess the suitability of different types of blood samples for measuring circulating 5-HT in clinical studies in canine species. Second, to further investigate the relationship between the serotonergic system and canine aggression. To this end, the assessment of 5-HT was simultaneously carried out in serum, plasma, and platelets of aggressive and nonaggressive dogs.

Materials and methods

Aggressive animals

Two Spanish veterinary teaching hospitals (Universidad de Zaragoza and Cardenal Herrera-CEU, Valencia) contributed to the collection of cases. Dogs included in the present study were referred to the Companion Animal Behavior Services within the respective hospitals owing to problems of aggression toward people and/or other dogs. Displaying any other type of behavioral problem did not constitute an exclusion criterion. Animals showing an underlying causative medical condition to the problem of aggression were excluded from the study.

Twenty males (17 entire and 3 castrated) and 8 females (7 entire and 1 spayed) were included in the aggressive group. The mean age was 4.2 years (ranging from 7 months to 13 years). The group consisted of 17 dogs of different breeds (4 English cocker spaniels, 2 German shepherds, 2 Rottweilers, 1 Alaskan malamute, 1 American pit bull terrier, 1 English bull terrier, 1 Catalan sheepdog, 1 Lhasa Apso, 1 Poodle, 1 Teckel, 1 West Highland white terrier, and 1 Yorkshire terrier) and 11 crossbreeds and mongrels.

Diagnosis of aggression was carried out by means of a detailed standard questionnaire on the dogs’ behavior and daily routine. Clinical classification of aggression was established in accordance with 3 main diagnostic criteria: target, context, and dog’s communicative signals (based on Fatjó et al., 2007). 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.

According to the main diagnosis, 2 animals showed intraspecific aggression, whereas the rest (n = 26, 93%) showed aggression toward humans, mainly directed to the family members during conflict or competitive situations (69.2%). Several forms of aggression were present in 39.3% of the animals.

Control animals

The control group was made up of 10 beagles (5 entire males, 3 entire females, and 2 spayed females) owned by the Universidad Cardenal Herrera-CEU for research purposes. The mean age was 3.1 years (ranging from 1 to 6 years). All these dogs were healthy and lacked any history of aggression toward people and/or other dogs. The care and use of animals followed the European guidelines (European Union Directive 86/609/EEC, 1986).

Sample collection and preparation

Blood drawn (3 mL) into ethylenediaminetetraacetic acid tubes was centrifuged at room temperature for 12 minutes at 152 × g to obtain platelet-rich plasma (PRP). The PRP supernatant was then transferred to another tube for platelet pellet preparation (according to the protocol by IBL GmbH, 1998; see later in the text). The ethylenediaminetetraacetic acid tubes were then centrifuged at 4,500 × g for 10 minutes at 4 °C to obtain plasma. Blood drawn (3 mL) into anticoagulant-free tubes was centrifuged at 4,500 × g for 10 minutes at 4 °C to obtain serum. Aliquots of plasma and serum were frozen and stored at −80 °C until the day of the analysis.

Platelet count was carried out in PRP (20 μL) using a Neubauer counting chamber. To obtain the platelet pellet, an aliquot of 200 μL of PRP was added to 800 μL of physiological saline and centrifuged at 4,500 × g for 10 minutes at 4 °C. The supernatant was discarded and 200 μL of double-distilled water was then added to the pellet. Aliquots were stored at −80 °C. On the day of the analysis, the samples were centrifuged at 10,000 × g for 2 minutes at room temperature after thawing the frozen aliquots.

Serotonin analysis

Serotonin was measured in duplicate with a commercial enzyme immunoassay technique (Serotonin-ELISA, DLD Diagnostika GmbH, Hamburg, Germany). The intra-assay and interassay coefficients of variations were 3.9%-5.4% and 6%, respectively. Concentrations of 5-HT were expressed in ng/mL (serum and plasma) and ng/10⁸ platelets (platelets).

Statistical analysis

A unifactorial multivariate analysis of variance was carried out to assess the effect of the factor “aggression” on
the concentrations of 5-HT in the 3 types of samples, namely serum, plasma, and platelets. Correlations were analyzed using the Pearson test.

Calculations were carried out using the statistical program SPSS 14.0. for Windows (SPSS, Inc, Chicago, IL). A \( P < 0.05 \) denoted statistical significance.

### Results

The unifactorial multivariate analysis of variance showed a significant effect of the factor “aggression” on 5-HT concentrations \( (P < 0.01) \). Aggressive animals showed lower 5-HT concentrations in serum, plasma, and platelets than nonaggressive animals. The higher mean 5-HT concentration was detected in serum samples. Serotonin results according to the type of sample are summarized in Table.

Serotonin concentration in platelets was positively correlated with that in serum \( (r = 0.465; \ P < 0.01) \) and plasma \( (r = 0.468; \ P < 0.01) \). In addition, 5-HT concentrations in serum and plasma were positively correlated \( (r = 0.489; \ P < 0.01) \).

### Discussion

In the present study, the assessment of 5-HT in serum, plasma, and platelets was carried out both in aggressive and nonaggressive dogs. Regardless of the type of sample, aggressive dogs showed significantly lower concentrations of circulating 5-HT than nonaggressive dogs. This finding agrees with previous studies that found an association between high aggression or impulsivity and low concentrations of CSF 5-hydroxyindoleacetic acid and circulating 5-HT in primates \( (\text{CSF}: \text{Melhman et al., 1994; Stanley et al., 2000; Soderstrom et al., 2001; Howell et al., 2007}; \text{platelets: Goveas et al., 2004}) \) as well as in dogs \( (\text{CSF: Reisner et al., 1996; serum: Çakiroğlu et al., 2007; Rosado et al., 2010a}) \).

Several mechanisms regulate the functioning of the 5-HT system in the brain: \( (i) \) 5-HT synthesis from tryptophan and degradation, \( (ii) \) 5-HT reuptake from synaptic cleft, and \( (iii) \) the density and sensitivity of 5-HT receptors \( (\text{Popova, 2006}) \). An alteration in any of these mechanisms may be the underlying reason for the reduced activity of the serotonergic system in aggression. Regarding canine aggression, an elevated plasma concentration of tryptophan was detected in a group of aggressive dogs in comparison with a nonaggressive group \( (\text{Juhr et al., 2005}) \). In addition, the administration of tryptophan-supplemented low-protein diets has been shown to be effective in reducing dominance aggression in dogs \( (\text{DeNapoli et al., 2000}) \). Moreover, a recent study by the authors found a higher platelet 5-HT uptake in aggressive dogs than in nonaggressive ones \( (\text{Rosado et al., 2010b}) \). Finally, a series of brain imaging studies showed an increase in the uptake of the 5-HT2A radioligand in all cortical areas of impulsive aggressive dogs compared with normal dogs \( (\text{Peremans et al., 2002, 2003, 2005}) \). All of these findings point toward an altered function of the serotonergic system in canine aggression.

In the present study, the mean concentration of 5-HT in the assayed samples reflected the normal distribution of blood 5-HT, with the higher concentration in serum, followed by platelets, and then, finally, plasma. Circulating plasma 5-HT is taken up by platelets, and is accumulated during the platelet’s lifecycle in dense granules. Thus, under normal conditions, 5-HT is almost entirely confined in platelets, whereas its concentration in plasma and other blood cells would be very low \( (\text{Ortiz et al., 1988; Kema et al., 2000}) \). Mück-Seler et al. \( (2002) \) have suggested that serum 5-HT concentration might indirectly represent a sum of platelet and plasma 5-HT concentrations. In the present study, the mean serum 5-HT concentration in each group of dogs \( (\text{i.e., aggressive and control groups}) \) was approximately the sum of the mean \( (\text{estimated}) \) platelet and plasma 5-HT concentrations.

Platelet and plasma 5-HT concentrations in the control group were similar to those reported by several studies on canine species \( \text{(platelets: Meyers et al., 1982; Aranda et al., 1994; plasma: Haga et al., 1996)} \). Likewise, serum 5-HT concentration was also similar to previously published data from whole blood in dogs \( (\text{Ferrara et al., 1987; LaRosa et al., 1989; Chen et al., 1993}) \). It is worth mentioning, however, the striking difference in serum 5-HT concentrations in the present study as compared with those in the study by Çakiroğlu et al. \( (2007) \), where relatively lower concentrations were detected both in nonaggressive and aggressive dogs. This difference may be explained in terms of methodological differences in measuring serum 5-HT \( (\text{e.g., the use of different enzyme-linked immunosorbent assay kits}) \).

A statistically significant difference in 5-HT concentration between aggressive and nonaggressive dogs was noted both in serum and platelets as well as in plasma samples. This indicates that any of them may represent a valid blood

<table>
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<th>Mean (SE) concentrations of serotonin in serum, plasma, and platelets of aggressive ((n = 28)) and control nonaggressive dogs ((n = 10))</th>
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<tr>
<td>Serotonin</td>
<td>Group</td>
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<tr>
<td>Serum (ng/mL)</td>
<td>Aggressive</td>
</tr>
<tr>
<td>Control</td>
<td>282.5 (28.1)</td>
</tr>
<tr>
<td>Plasma (ng/mL)</td>
<td>Aggressive</td>
</tr>
<tr>
<td>Control</td>
<td>51.8 (6.7)</td>
</tr>
<tr>
<td>Platelets (ng/10^8 plts)</td>
<td>Aggressive</td>
</tr>
<tr>
<td>Control</td>
<td>90.8 (10.7)</td>
</tr>
</tbody>
</table>
sample for measuring circulating 5-HT to detect differences between both groups. The positive correlation found among the 3 types of samples may also support this suggestion. The choice of either one of these different types of blood samples for clinical applications, however, deserves some comments. Plasma is easy to obtain, but, as previously mentioned, 5-HT concentrations in this pool are low, and therefore, more sensitive techniques are required to accurately detect the amine (e.g., high-performance liquid chromatography). In addition, plasma may be potentially contaminated with platelet 5-HT (Anderson et al., 1987).

Finally, platelet preparation is an arduous task, as an additional platelet count is necessary to express the concentration of 5-HT apart from blood centrifugation to obtain PRP. Because serum 5-HT concentration is considered an indirect measure of platelet 5-HT content, it could be suggested that serum is the easiest sample type to assess circulating 5-HT in dogs. Nevertheless, the assessment of plasma 5-HT may be of great interest also in the field of research on the serotonergic system in canine aggression. It is suggested that plasma 5-HT is a rapid turnover pool affected by short-term changes in the 5-HT physiology (e.g., synthesis, inactivation, uptake), whereas platelet 5-HT is a reserve, slow turnover pool influenced by sustained changes. Thus, the measurement of plasma or platelet (serum) 5-HT concentrations would be useful to study different aspects of 5-HT physiology (Ortiz et al., 1988).

One limitation to the present work is the use of an experimental cohort of beagles as control subjects for a group of aggressive pet dogs belonging to different breeds. Despite their regular contact with humans, the rearing and environmental conditions in the group comprising beagles clearly differed from those dogs in the pet group, and this may have affected the activity of the 5-HT system even early in life. In addition, it is possible that breed-related differences may occur in the 5-HT system functioning. It is important to note, however, that the use of experimental beagles as a control group might provide some advantages such as low individual variability (high group homogeneity) and, therefore, high reproducibility of the experiment in other laboratory settings. Another limitation of the study is that the 2 study groups were not matched for sex and reproductive status, with proportionally more entire males in the aggressive than the control group, and there is evidence that testosterone (but also estradiol) interacts with the 5-HT system to influence the likelihood of aggression, probably by modulating 5-HT receptors (Nelson and Chiavegatto, 2001; Soma et al., 2008). In another published study with a larger sample of dogs, however, we did not find a significant effect of sex on serum 5-HT levels (Rosado et al., 2010a). Finally, it is important to note that the aforementioned limitations would have not affected the first aim of the present study, that is, to assess and compare the suitability of different types of blood samples for measuring circulating 5-HT in dogs. Anyway, future studies on this field should consider breed, sex (and reproductive status), as well as age-matched groups to assess these possible influencing factors.

In conclusion, the present results may suggest an inverse relationship between canine aggression and the activity of the serotonergic system. To the authors’ best of knowledge, this is the first study to simultaneously assess the concentration of 5-HT in serum, plasma, and platelets in aggressive and nonaggressive dogs. The 3 assayed blood samples showed differences between both groups, but considering the simplicity of the methodology, the authors propose serum as the more suitable sample for measuring circulating 5-HT in clinical studies in dogs. The determination of circulating 5-HT may have important applications for behavior medicine in the future. For example, it could be used for deciding which aggressive dogs might benefit from drug therapy and for monitoring the response to these therapies. In addition, it could be used for research purposes with the aim of creating new tools for diagnosis and treatment of canine aggression.

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References


