

Dehydroepiandrosterone and cortisol concentrations in the cerebrospinal fluid of dogs

Paolo Mongillo^{1,*}, Marco Bernardini^{2,3}, Elisa Prana¹, Federica Balducci³,
Gianfranco Gabai¹, Lieta Marinelli¹

Departments of¹Comparative Biomedicine and Food Sciences,

²Animal Medicine, Production and Health, University of Padova, Legnaro 35020, Italy

³Portoni Rossi Veterinary Hospital, Zola Predosa 40069, Italy

(Received: November 9, 2016; Revised: January 12, 2017; Accepted: January 19, 2017)

Abstract: Concentrations of cortisol, dehydroepiandrosterone and dehydroepiandrosterone-sulfate were measured by performing radioimmunoassay of the cerebrospinal fluid of 68 dogs diagnosed with idiopathic epilepsy or inflammatory, degenerative, or non-neurological disease. No steroid concentration differences were found among diagnoses. Dehydroepiandrosterone and dehydroepiandrosterone-sulfate concentrations were higher in males than in females and dehydroepiandrosterone-sulfate decreased with increasing age. No sex or age effects were observed on cortisol or hormone ratios. Although limited to a relatively small sample, our results show sex- and age-dependent variations in these neurosteroid concentrations in cerebrospinal fluid. The role of such variations in the pathophysiology of the dog brain warrants further investigation.

Keywords: cerebrospinal fluid, dehydroepiandrosterone, dogs, hydrocortisone, sex

In humans, plasma levels of dehydroepiandrosterone (DHEA) and its circulating storage form DHEA sulphate (DHEA-S) are dependent on sex, higher concentrations being found in adult men than women. In both sexes, however, concentrations decline during ageing, falling to 5-30% of their peak value by the eighth decade [8]. A similar age-related decline is also observed in the cerebrospinal fluid (CSF), but there seems to be no difference between sexes at this level [6]. Since cortisol levels increase with ageing, higher cortisol/DHEA ratios are found in both plasma and CSF of elderly people [6]. In adult dogs, plasma DHEA is much higher in males than in females and only in the latter there is a clear age-related decline; at the same time, no age-related differences have been found for plasma cortisol concentrations in this species [12]. The dog's body size can affect steroid concentrations, as higher plasma cortisol concentrations have been observed in small than in large dogs [13].

Since the first description of its synthesis in the central nervous system (CNS) [1], interest in DHEA has grown in view of its role as a neuroactive steroid. DHEA exerts neurotrophic and neuroprotective functions, antagonizing detrimental effects of glucocorticoids, whose concentrations in both blood and CSF have been correlated with functional and morphological neural deficits [9, 16]. The ratio between cortisol and DHEA

and DHEA-S concentrations is therefore considered crucial in the pathophysiology of neurodegenerative processes and other neurologic diseases. For instance, decreased DHEA-S and DHEA-S to cortisol ratio have been associated with age-related neurodegeneration and cognitive decline and epilepsy [4, 5]. Conversely, the concentrations of both DHEA-S and cortisol increase in patients with inflammatory neurological diseases, compared to non-inflammatory ones [7].

Dogs develop age-related lesions and functional deficits of the CNS analogous to those of humans and are considered a good model of age-related neurodegenerative processes [15]. A syndrome characterized by abnormal behaviours and believed to be consequence of such neurodegeneration has been described in aged dogs [15]. Dogs have also been proposed as models for other neurological diseases, including epilepsy as well as neurodegenerative and inflammatory neurological conditions [2, 11, 14], in which a role of cortisol, DHEA and DHEA-S could be hypothesized. The study of DHEA and cortisol in the pathophysiology of canine CNS is therefore relevant to both veterinary and human medicines. To the best of our knowledge there is no data on these steroids' concentrations in canine CSF, which is what this study aimed at investigating. Given the previous indications from both the canine and human literatures, we investigated sex

*Corresponding author

Tel: +39-49-641862, Fax: +39-49-641174

E-mail: paolo.mongillo@unipd.it

and age as the main factors potentially affecting the hormones concentrations in the CSF. However, considered the potential role of these hormones in the pathophysiology of diverse neurological conditions, we included the dog neurological characterization as a further factor of interest.

Concentrations of DHEA, DHEA-S and cortisol were measured in CSF obtained from 49 male dogs (all of which were sexually intact) and 19 female dogs (including 9 intact subjects), presented in two referral veterinary hospitals over a period of two years. For the aims of this study, dogs were classified as of small (< 20 kg, $n = 30$) or large size (≥ 20 kg, $n = 38$). Samples were collected from the cisterna magna ($n = 55$) or the cisterna lumbalis ($n = 13$) from dogs euthanized for non-neurological diseases, immediately after the procedure and from dogs for which CSF collection was part of the diagnostic workup. For the latter, CSF was collected under anaesthesia, obtained through intravenous induction and maintained with volatile anaesthetics; the specific anaesthetic protocol for each dog was chosen and monitored by a veterinary anaesthesiologist. Only dogs that were not under glucocorticoid treatment were included in the study. The history, clinical signs, neurological evaluation, CSF differential white cell count and other diagnostic tests (magnetic resonance imaging, radiography, serology, cytology, electrodiagnostic studies and surgical findings), were used to assign dogs to one of the following diagnostic categories: idiopathic epilepsy ($n = 16$; mean age \pm SD, 4.5 ± 2.4 years), inflammatory- ($n = 16$, 5.9 ± 3.8 years), degenerative- ($n = 15$, 8.7 ± 2.6 years), or non-neurological- disease ($n = 20$, 6.6 ± 5.1 years).

Collected CSF was visually inspected for blood contamination; non-contaminated samples were immediately frozen and stored at -20°C until assayed. DHEA and cortisol were measured with a solid-phase radioimmunoassay (RIA) as previously described [12]. DHEA-S concentrations were analysed by a solid-phase RIA [10], after double extraction with ethyl ether:hexane mixture (90:10) and chloroform:2-butanol mixture (50:50). The dry extracts were carefully dissolved in a proper volume of buffer (phosphate-buffered saline, 0.1% bovine serum albumin, pH 7.4) in order to achieve concen-

trations that fell in the linear part of standards curves. The subsequent RIA procedures were performed in duplicate. The detection limits of the assays, as calculated by RiaSmart software (PerkinElmer Life and Analytical Sciences, USA), were 0.008 pmol/well, 0.005 pmol/well and 0.004 pmol/well for CSF cortisol, DHEA and DHEA-S, respectively. CSF cortisol, DHEA and DHEA-S assays were validated for the canine species through parallelism, precision and recovery tests (Table 1).

Correlations were computed between DHEA, DHEA-S and cortisol concentrations in the CSF. A general linear model was used to evaluate the effect of factors like diagnostic category, size, sex, and the gonadectomy (sex) nested term, and of covariate age on steroids' concentration and their ratios in CSF. Due to inherent characteristics of the neurological conditions, which prevalence may vary in relation to the dogs' age and sex, and the fact that some categories of dogs (e.g. castrated males) are very rarely found in Italy, obtainment of a balanced sample was not achievable. This issue was soothed by using Type IV sum of square methods for the calculation of the model statistics, as it allows to overcome problems of empty cells in the dataset. All variables were log-transformed to achieve normal distribution. SPSS was used for statistical analysis (IBM SPSS Statistics V22.0.0; IBM, USA).

In the CSF, concentrations of DHEA and DHEA-S were correlated ($r = 0.36$, $p = 0.002$) while no correlation was found between cortisol and either DHEA ($r = -0.025$, $p = 0.88$) or DHEA-S ($r = -0.028$, $p = 0.84$). The dog's size had no effect on any of the steroids' concentrations or ratios ($p > 0.5$ in all cases). Diagnostic category had no effect on any of the steroids' concentrations or ratios ($p > 0.5$ in all cases); however, limited group sizes reduced the power of the analysis, and no conclusive consideration can be made on role of DHEA and cortisol in these pathologies. Age had had a significant effect on DHEA-S, which decreased with increasing age ($F = 5.138$, $p = 0.028$); no age effect was found on DHEA and cortisol levels, or the cortisol/DHEA and cortisol/DHEA-S ratios in the CSF ($p > 0.2$ in all cases). Sex [but not the gonadectomy (sex) term] was the main factor affecting

Table 1. Validation parameters of hormonal assays in canine cerebrospinal fluid

Hormone	Parallelism	R^2	Recovery	R^2	Intra-assay	Inter-assay
DHEA-S	$y = 0.099x + 0.004$	0.99	$y = 0.96x + 3.63$	0.98	0.9%	9.9%
DHEA	$y = 0.205x + 0.005$	0.99	$y = 1.18x + 0.01$	0.99	5.9%	10.0%
Cortisol	$y = 19.06x + 0.34$	0.99	$y = 0.96x + 0.15$	0.99	7.8%	11.5%

Parallelism was expressed as the regression curve between observed hormone concentrations and the reciprocal of the dilution factors of serially diluted samples ($H_{\text{obs}} = a \times 1/\text{df} + b$). In all assays, values of intercept b were not significantly different from zero indicating a good degree of parallelism. Recovery was expressed as the regression curve between observed and expected hormone concentrations ($H_{\text{obs}} = a(H_{\text{exp}}) + b$) measured in cerebrospinal fluid (CSF) samples spiked with known amounts of dehydroepiandrosterone (DHEA; final CSF concentration range, 0.09–0.69 nmol/L), DHEA sulphate (DHEA-S; final CSF concentration range, 0.03–0.27 nmol/L) or cortisol (final CSF concentration range, 1.66–55.25 nmol/L). In all cases, angular coefficient a was not different from 1 and intercept b was not different from zero, indicating good correspondence between observed and expected concentrations. The results of the intra- and inter-assay precision test are expressed as coefficients of variation.

Table 2. Mean \pm SD of molar concentrations (nmol/L) of DHEA, DHEA-S, cortisol and their ratios in the cerebrospinal fluid of male and female dogs. General linear model, Type IV sum of squares

Hormone or hormonal ratio	Males	Females	<i>F</i>	<i>p</i> value
DHEA	0.33 \pm 0.30	0.13 \pm 0.08	8.154	0.007
DHEA-S	0.10 \pm 0.16	0.04 \pm 0.01	7.215	0.010
Cortisol	6.2 \pm 7.8	7.6 \pm 7.5	0.004	0.951
Cortisol/DHEA	27.9 \pm 38.2	118.6 \pm 189.5	0.366	0.554
Cortisol/DEHA-S	85.2 \pm 116.1	235.2 \pm 249.1	0.384	0.541
DHEA-S/DHEA	0.30 \pm 0.32	0.39 \pm 0.46	0.380	0.540

DHEA and DHEA-S concentrations in the CSF, which were both approximately twice as high in males than females (Table 2). The cortisol/DHEA and cortisol/DHEA-S ratio did not show a statistically significant difference between our male and female dogs (Table 2).

In this study, we report for the first time the concentrations of cortisol, DHEA and DHEA-S in the CSF of dogs. Cortisol concentrations were only slightly lower than those reported in humans; larger differences were found between our dogs and humans in DHEA and DHEA-S concentrations [6], as already noted for these steroids' plasma concentrations [3]. Our dogs' steroids concentrations also showed a higher variability than that reported for humans [6]. Since sampling was performed under anaesthesia, such variability cannot represent an effect of contextual stimuli (*e.g.* physical activity, emotional condition, sampling procedures), and is likely to reflect actual individual variability.

Age negatively affected the concentration of DHEA-S in the CSF. Several hypotheses can be made about the mechanisms responsible for such reduction, including an age-related decrease in plasma DHEA-S, a reduced transport across the blood-brain barrier, or a reduced local synthesis of DHEA-S in the CNS of aged dogs. While our data does not allow to speculate on the underlying mechanism, it should be noted that even in humans, in spite of greater age-associated changes in CSF concentrations of neurosteroids [6]. The mechanisms leading to such changes have not yet been understood.

As regards the effect of sex, we observed a higher concentration of DHEA and DEHA-S in the CSF of male dogs. In humans, where the adrenals represent the main source of DHEA [6], the same differences are not found. In dogs, however, the hormone is thought to be mainly synthesized in the testis [12], which likely explains the large sex-related differences observed in the present study. At the same time, the lack of differences in the cortisol/DHEA and cortisol/DHEA-S ratios suggests that the relative concentrations of the two hormones are similarly regulated in the CSF of male and female dogs. However, cortisol and, consequently, both cortisol/DHEA and cortisol/DHEA-S ratios showed a large variability, which can explain the lack of statistically significant differences between sexes; a look at the mean values of the cortisol/DHEA and the cortisol/DEHA-S ratios in females and males still warrants the possibility that a different bal-

ance of these hormones exists in the two sexes.

In conclusion, results of this study represent a novel finding, showing age-related changes and large sex-based differences in the concentrations of neuroactive steroids in canine CSF. In view of the antagonistic functions of DHEA (and DHEA-S) and cortisol and their role in brain physiopathology, the role of such variations warrants further investigation.

Acknowledgments

This study was funded by the University of Padova (fund code: CPDA144871/14). Dr. Elisa Prana received a PhD grant from the University of Padua. We would like to thank Laura Da Dalt and Tommaso Brogin for their valuable technical support.

References

1. **Baulieu EE.** Steroid Hormones in the brain: several mechanisms? In: Fuxe K, Gustafsson JA, Wetterberg L (eds.). Steroid Hormone Regulation of the Brain. pp. 3-14, Pergamon, Oxford, 1981.
2. **Coates JR, Winger FA.** Canine degenerative myelopathy. Vet Clin North Am Small Anim 2010, **405**, 929-950.
3. **Cutler GB Jr, Glenn M, Bush M, Hodgen GD, Graham CE, Loriaux DL.** Adrenarache: a survey of rodents, domestic animals, and primates. Endocrinology 1978, **103**, 2112-2118.
4. **Ferrari E, Cravello L, Muzzoni B, Casarotti D, Paltro M, Solerte SB, Fioravanti M, Cuzzoni G, Pontiggia B, Magri F.** Age-related changes of the hypothalamic-pituitary-adrenal axis: pathophysiological correlates. Eur J Endocrinol 2001, **144**, 319-329.
5. **Galimberti CA, Magri F, Copello F, Arbasino C, Cravello L, Casu M, Patrone V, Murialdo G.** Seizure frequency and cortisol and dehydroepiandrosterone sulfate (DHEAS) levels in women with epilepsy receiving antiepileptic drug treatment. Epilepsia 2005, **46**, 517-523.
6. **Guazzo EP, Kirkpatrick PJ, Goodyer IM, Shiers HM, Herbert J.** Cortisol, dehydroepiandrosterone (DHEA), and DHEA sulfate in the cerebrospinal fluid of man: relation to blood levels and the effects of age. J Clin Endocrinol Metab 2000, **81**, 3951-3860.
7. **Heidbrink C, Häusler SFM, Buttman M, Ossadnik M, Strik HM, Keller A, Buck D, Verbraak E, van Meurs M, Krockenberger M, Mehling M, Mittelbronn M, Laman JD, Wiendl H, Wischhusen J.** Reduced cortisol levels in

- cerebrospinal fluid and differential distribution of 11β -hydroxysteroid dehydrogenases in multiple sclerosis: implications for lesion pathogenesis. *Brain Behav Immun* 2010, **24**, 975-984.
8. **Laughlin GA, Barrett-Connor E.** Sexual dimorphism in the influence of advanced aging on adrenal hormone levels: the Rancho Bernardo Study. *J Clin Endocrinol Metab* 2000, **85**, 3561-3568.
 9. **Maninger N, Wolkowitz OM, Reus VI, Epel ES, Mellon SH.** Neurobiological and neuropsychiatric effects of dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS). *Front Neuroendocrinol* 2009, **30**, 65-91.
 10. **Marinelli L, Trevisi E, Da Dalt L, Merlo M, Bertoni G, Gabai G.** Dehydroepiandrosterone secretion in dairy cattle is episodic and unaffected by ACTH stimulation. *J Endocrinol* 2007, **194**, 627-635.
 11. **Melo GD, Seraguci TF, Schweigert A, Silva JES, Grano FG, Peiró JR, Lima VMF, Machado GF.** Pro-inflammatory cytokines predominate in the brains of dogs with visceral leishmaniasis: a natural model of neuroinflammation during systemic parasitic infection. *Vet Parasitol* 2013, **192**, 57-66.
 12. **Mongillo P, Prana E, Gabai G, Bertotto D, Marinelli L.** Effect of age and sex on plasma cortisol and dehydroepiandrosterone concentrations in the dog (*Canis familiaris*). *Res Vet Sci* 2014, **96**, 33-38.
 13. **Reimers TJ, Lawler DF, Sutaria PM, Correa MT, Erb HN.** Effects of age, sex, and body size on serum concentrations of thyroid and adrenocortical hormones in dogs. *Am J Vet Res* 1990, **51**, 454-457.
 14. **Steinmetz S, Tipold A, Löscher W.** Epilepsy after head injury in dogs: a natural model of posttraumatic epilepsy. *Epilepsia* 2013, **544**, 580-588.
 15. **Studzinski CM, Araujo JA, Milgram NW.** The canine model of human cognitive aging and dementia: pharmacological validity of the model for assessment of human cognitive-enhancing drugs. *Prog Neuropsychopharmacol Biol Psychiatry* 2005, **29**, 489-498.
 16. **Wolf OT, Kirschbaum C.** Actions of dehydroepiandrosterone and its sulfate in the central nervous system: effects on cognition and emotion in animals and humans. *Brain Res Rev* 1999, **30**, 264-288.