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Characterisation of transthyretin and retinol-binding protein in plasma and cerebrospinal fluid of dogs

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Abstract

The aim of this study was to investigate differences in concentrations of vitamin A, transthyretin (TTR) and retinol-binding protein (RBP) between plasma and cerebrospinal fluid (CSF) in dogs. RBP was detected using ELISA, and both RBP and TTR by Western blot analysis after separation on SDS–PAGE. Vitamin A was determined by high performance liquid chromatography.

RBP and TTR as well as vitamin A were detected in all samples but at substantially lower concentrations in CSF compared to plasma. RBP in dog plasma showed a similar molecular mass to that of humans, whereas canine TTR had a lower molecular mass. Comparison between plasma and CSF showed that both RBP and TTR were of lower molecular mass in CSF. In CSF, RBP and retinol were present at 10–100-fold lower concentrations compared to plasma. Retinyl esters were present only in minute amounts in 5/17 samples. In conclusion, the CSF of dogs compared to humans is significantly different in terms of both quality and quantity of transport proteins for vitamin A.
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1. Introduction

The choroid plexus constitutes the blood-cerebrospinal fluid (CSF) barrier. The structural and functional integrity of this barrier is crucial to the homeostasis of the internal milieu of the central nervous system (CNS) (Chodobski and Szmydynger-Chodobska, 2001; Segal, 2001), for which reason the impairment of the choroid plexus may quite possibly be associated with certain neurological diseases (Reiber, 2003). The regulatory functions of the choroid plexus are accomplished by two mechanisms: the selective and limited access for blood-borne substances to the cerebral compartment and by synthesizing specific essential components for brain nutrition and integrity (Segal, 2001).

Along with various other proteins, transthyretin (TTR) and retinol-binding protein (RBP) are present in the CSF of different species (Aldred et al., 1995). TTR, formerly called prealbumin, belongs to a group of proteins including thyroxine-binding globulin and albumin that bind to and transport thyroid hormones in the blood. It is present in the plasma as a tetramer of non-covalently bound monomers of 127 amino acids (~13,800 Da). Whereas plasma TTR originates primarily in the liver, brain TTR originates exclusively in the choroid plexus (Ingenbleek and Young, 1994).

In addition to TTR, the choroid plexus produces and secretes RBP into the CSF (Aldred et al., 1995). The lipocalin RBP is a low molecular weight (~21,000 Da) transport protein for retinol in plasma. Both RBP and

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TTR are involved in the transport of retinol in plasma and CSF. Retinol is transported by RBP across the blood-CSF barrier to the CNS and local TTR co-operates in this transport of retinol (Zheng et al., 2001).

In neuronal structures, retinol and its derivates, the retinoids, are important components involved in embryonic developments as well as other important functions in later life (Blomhoff, 2002). Specific neurological disorders such as schizophrenia and Alzheimer's disease may be associated with a defect in retinoid transport and/or metabolism (Goodman and Pardee, 2003). Investigations looking at aspects of retinoid transport in the CSF have been conducted in species in which plasma retinol levels are tightly and homeostatically regulated (Warner et al., 2002; Zheng et al., 2001). In the dog, however, in addition to retinol, substantial amounts of retinyl esters are present in blood plasma, influenced by dietary vitamin A intake (Schweigert and Bok, 2000).

The present study was conducted to describe possible quantitative and qualitative differences in retinol and retinyl esters, as well as transport proteins RBP and TTR in plasma and CSF of dogs. It was hoped that this would provide further information on the peculiarities of vitamin A metabolism in dogs, and aid in the future evaluation of the possible involvement of these components in various neurological disorders.

2. Materials and methods

Paired CSF and plasma samples ($N = 17$) were obtained from dogs with thoraco-lumbal discopathy grade I (pain only, no neurological deficits, but resistant to medical therapy). Puncture of the cerebellomedullary cistern (CMC) was performed for diagnostic purposes in order to exclude infectious disorders of the CNS. The animals were treated at the Veterinary Clinic for Small Animals of the Free University of Berlin, Germany.

Collection from the CMC was performed in lateral recumbency under general anaesthesia (propofol 4 mg/kg KM, Rapinovet). The first 0.5–1 mL were collected into a sterile vial without anticoagulant. The CSF samples were centrifuged at 2000*g* for 10 min to eliminate cells and other insoluble material. A blood sample was taken at the same time from the cephalic vein and placed in EDTA-coated, evacuated tubes. Plasma was prepared by centrifuging blood samples (1500*g*, 10 min at 4 °C). Aliquots of CSF and plasma were frozen at −80 °C within 30 min of collection. All analyses were performed within one month. Cell counting was performed in a Fuchs–Rosenthal chamber. The number of RBC per microliter of CSF was used as a measure of blood contamination during CSF collection. Red blood cells lyse rapidly within CSF, resulting in CSF xanthochromia; therefore, the presence of intact RBC in CSF in the absence of CSF xanthochromia indicates iatrogenic, rather than disease-induced, haemorrhage into the subarachnoid space. The inclusion criteria for the study were a normal white-cell count (<4/μL), no iatrogenic blood contamination during CSF collection (RBC 0–18/μL) and no evidence of CSF xanthochromia. A protein elevation was detected by Pandy reaction and by a silver stained SDS–PAGE. In the case of intrathecal IgG production, samples were excluded. Furthermore, an albumin quotient (AQ) from CSF to plasma was determined.

2.1. Determination of retinol and retinyl esters

Retinol and retinyl esters in plasma and CSF were determined using a modified high performance liquid chromatography (HPLC) method, as described elsewhere (Schweigert et al., 2000). Briefly, 500 μL CSF or 200 μL plasma were deproteinised with 500 μL ethanol and extracted twice with *n*-hexane (1 mL each time stabilised with 0.05% butylated hydroxytoluene) and vortexed for 5 min. The supernatants were pooled, evaporated under nitrogen, reconstituted in 200 μL isopropanol and injected into the HPLC-system (Waters). For the separation of the compounds, a C30 carotenoid column, (5 μm, 250 × 3 mm; YMC) with a solvent system consisting of solvent A with methanol/water (90/10; v/v, with 0.4 g/L ammonium acetate in H_2O) and solvent B with methanol/methyl-*tert*-butyl-ether/water (8:90:2; v/v/v, with 0.1 g/L ammonium acetate in H_2O) was applied. For detection and characterisation, a photodiode array detector was used (Model 996, Waters). Retinol and retinyl esters were quantified by measuring the absorption at 325 nm using retinol as an external standard (Sigma).

2.2. Determination of TTR and RBP using Western blotting

The samples (5 μL) were electrophoresed through 12% sodium dodecyl–polyacrylamide gel electrophoresis (SDS–PAGE) using the buffer system of Laemmli (1970). After SDS–PAGE, the proteins were transferred from the gel onto a polyvinylidene diflouride (PVDF) membrane (Millipore) for 30 min, and blocked with 5% milk powder in Tris buffered saline, 0.1% Tween 20 (TBST, pH 7.6) for 1 h. The membranes were then incubated for 90 min with 1:300 diluted crossreacting rabbit anti-human RBP or rabbit anti-human TTR (DakoCytomation). After washing with 0.3% TBST, the membranes were incubated with 1:500 diluted peroxidase conjugated sheep anti-rabbit IgG (DakoCytomation) for 1 h. The colour reaction was developed using Luminol reaction (Chemiluminescence Blotting Sub-

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