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Severe cognitive impairment correlates with higher cerebrospinal fluid levels of lactate and pyruvate in a canine model of senile dementia

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Abstract

Diagnosis of dementia of the Alzheimer's type depends on clinical criteria and exclusion of other disorders because, at this time, a validated biological marker, aside from histological brain examination, remains to be established. The canine counterpart of senile dementia of the Alzheimer type (ccSDAT) is considered a promising model for examining behavioral, cellular and molecular processes involved in early phases of human brain aging and Alzheimer disease (AD). In order to investigate the first events taking place in canine cognitive dysfunction, in this paper we established a new and rapid behavioral test that finely discriminates the degrees of cognitive impairment. Cerebrospinal fluid (CSF) analysis was performed to determine the relationship between each disease stage and modification of cerebral energy metabolism. Our results demonstrate a parallel increase of lactate, pyruvate and potassium concentrations in the severe cognitive deficit. These differences are discussed in view of the neuroprotective role presently given to lactate.

Keywords: Cerebrospinal fluid; Cognitive test; Dog; Energy metabolism; Lactate; Potassium; Pyruvate

1. Introduction

Dementia of the Alzheimer's type is characterized by the combination of an appropriate clinical picture of progressive dementia and neuropathological confirmation from study of brain tissue. The diagnosis of "probable Alzheimer's disease" depends on clinical criteria alone, that is, a characteristic history, compatible findings on physical and mental status examination, and the exclusion of other disorders that mimic AD by appropriate information from history, examination and laboratory tests. Despite an explosion of relevant biologic research on AD, at this time, there is no validated biologic marker useful for the diagnosis of AD aside from findings on histological examination of brain tissue (Braak and Braak, 1991). Cerebrospinal fluid (CSF) levels of non-specific neuronal metabolic substrates such as pyruvate and lactate have been found increased in AD and vascular dementia (Parnetti et al., 2000). In addition, some elderly patients exhibit significant memory deficits but do not have impairments in everyday activities (Edwards et al., 2004; Van der Flier et al., 2004). These symptoms that are not always a precursor of AD cannot be easily differentiated from those that would progress to develop dementia (Bowen et al., 1997), and no specific

Abbreviations: AD, Alzheimer disease; ANS, astrocyte-neuron shuttle; ccSDAT, canine counterpart of senile dementia of the Alzheimer type; CNS, central nervous system; CSF, cerebrospinal fluid; MMSE, mini mental score evaluation; LCD, light cognitive deficits; SCD, severe cognitive deficits; YC, young control.

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information is presently available on differences regarding their biological characteristics (Assini et al., 2004; Blennow, 2004).

The canine counterpart of senile dementia of the Alzheimer type (ccSDAT) is considered a promising model for examining behavioral, cellular and molecular processes involved in early phases of human brain aging and AD because it develops with common features in human and canine brain (Cummings et al., 1993; Pugliese et al., 2004). The ccSDAT presents several AD hallmarks such as deterioration of cognitive function, plaques, congophilic angiopathy and neuronal loss (Adams et al., 2000). Canine preamyloid lesions contain substantial amounts of Aβ peptides found in human senile plaques (Wisniewski et al., 1996) resulting in four specific identifiable stages in which distribution is similar to the one observed in human (Cummings et al., 1993; Miyawaki et al., 2002; Pugliese et al., 2004; Satou et al., 1997). Because of that, dogs are quite unique to investigate the behavior and biological aspects of the early phases of AD, and to avoid the use of non-human primate models.

The brain is highly metabolically active and relies strongly on an efficient nutrient delivery, including, above all, the constant supply of glucose and oxygen. Neural cells not only do not store glycogen, they also do not execute gluconeogenesis. An increased nervous system activity requires increased energy metabolism in neurons, part of which will be used for restoring the Na^+–K^+ balance. In this situation, the neuronal energy metabolism would be fueled by oxidation of glucose and lactate taking place selectively in brain (Pellerin et al., 1998; Smith et al., 2003). This lactate would be delivered by astrocytes through the astrocyte-neuron shuttle (ANS), a major metabolic pathway in neural tissue (Gladden, 2004). So, nerve cell metabolic adaptations predict not only the size and complexity of brain structures, but also their longevity or resistance to failure. For example, in normal brain tissue, fluctuations in ion levels are limited, but an intense neuronal excitation, defective inhibition or energy failure, cause extracellular [K^+] to rise and [Na^+], [Ca^{2+}] to fall (Kimelberg et al., 1993). Ion levels are then driven beyond normal limits, and a positive feedback can develop, as abnormal ion distributions influence neuronal function which in turn aggravates ion altered distribution (Somjen, 2001). Since glial cells and neurons interact intensely, many of the neuronal alterations like the ones derived from energy failure are presently regarded as secondary to glial mischanges (Ramonet et al., 2004; Schousboe et al., 1997).

In this paper, a new instrument for the assessment of behavioral abnormalities of 25 dogs was designed to investigate discriminative events between early and late stages of ccSDAT. Based on the few existing clinical scales of memory disorders for veterinary practice (Colle et al., 2000; Kiatipattanasakul et al., 1996) and the Mini Mental Score Evaluation (MMSE) and Statistical Manual of Mental Disorders, 4th Edition (DSM IV) (Frances et al., 1994) that evaluate AD neuropsychological features, we developed a new test to characterize the canine global and progressive deterioration of memory, cognition and personality. Once filled out by the veterinarian interview of the owners, results were analyzed and compared with the data obtained directly from the clinical exploration of the dogs after validation of each item. Analysis of CSF parameters related to brain energy metabolism was also performed to determine the correlation between energy consumption, ion balance and cognitive deficit.

2. Methods

2.1. Animals

Twenty five dogs, eight females and seventeen males of different breeds and weight, and ranging from 1 to 16 years, were examined at the veterinary hospital *Ars Veterinaria*, Barcelona, Spain and used for this study (Table 1). In no case, dogs received previously any treatment that might interfere with their behavior. All the dogs were clinically evaluated by a veterinary (J.M.). Dogs presenting a specific central nervous system (CNS) alteration (tumor, inflammation, etc.) that could interfere with their cognitive status were not included in this study. Twenty of these dogs presented several main pathologies and were referred to the hospital with no evident clinical signs of behavioral deficit; five dogs presented signs of behavioral deficits as main pathology, clinically evaluated as mild (+), moderate (++), and marked (+++). The clinical signs of cognitive deficits included loss of established housetraining habits, disorientation in familiar surroundings, decreased activity and playfulness, vitality and interaction with owner, increased irritability, compulsive behavior and modifications of the sleep/awake cycle (Ruehl et al., 1995; Kiatipattanasakul et al., 1996; Colle et al., 2000; Pugliese et al., 2004).

All animals were treated according to European legislation on animal handling and experiments (86/609/EU) and procedures were approved by Ethical Committee of the University of Barcelona, Barcelona, Spain. All efforts were made to minimize animal suffering and to use no more than the number of animals needed for reliable scientific data.

2.2. Cognitive evaluation

To better evaluate the cognitive status of all 25 dogs a new cognitive test, adapted from veterinary tests of other authors (Colle et al., 2000; Kiatipattanasakul et al., 1996) and the MMSE and DSM IV, was used in collaboration with the pet owners. The test was filled out by a veterinarian (J.M.) and consisted initially of 16 items (Table 2).

For each item score 1 indicated the normality of the specific behavior, and scores 2, 3, 4 and 5 the degrees of abnormal behavior. The final total score reflected the cognitive status of the animal. In some cases normality