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SPUMIFORM BASEMENT MEMBRANE ABERRATIONS IN THE MICROVASCULATURE OF THE MIDBRAIN PERIAQUEDUCTAL GRAY REGION IN HAMSTER: ROSTRO-CAUDAL PATHOGENESIS?

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Abstract—Spumiform basement membrane degeneration (sbmd) is a specific kind of aberration present in the capillaries of the midbrain periaqueductal gray (PAG) region of the senescent hamster. These capillaries, separated by the ependymal cell layer, are bordering the Sylvian cerebral aqueduct. The aqueduct, connecting the 3rd and 4th ventricle, may be crucial for local homeostatic as well as general autonomic functions of the PAG. Local pressure effects of the flowing and pulsating cerebrospinal fluid on the PAG-vasculature are probably different for the rostral 'entrance' and the caudal 'exit' of the aqueduct. In view of the different functions of the various divisions of the PAG, the frequency and extent of the aberrations in the rostral, intermediate and caudal dl/vlPAG-microvasculature could shed some light on the causal factors involved in the regional distribution of the particular microvascular aberrations found in the PAG during aging. In the present study we investigated the ultrastructure of capillaries in dorsal and ventral subdivisions of anterior and posterior regions of the PAG of young and old female Syrian hamsters. Sbmds were classified into four stages of spumiform severity and for each stage the frequency was determined in the rostral PAG, at two levels in the intermediate PAG and in a dorsal and a ventral part of the caudal PAG.

Results of our quantitative studies showed that in aged hamster PAG various stages of sbmd were present in 91.6 ± 0.6% of all capillaries. No clear evidence was found for regional differentiation between rostral, intermediate and caudal parts of the PAG. Next to sbmd, capillary split basement membrane (sbm) and vacuolization were common features at all five PAG locations. 84.3 ± 2.3% of all screened PAG capillaries displayed sbm. In agreement with our previous findings, several other types of microvascular aberrations were observed in addition to general aspects of aging and some ependymal structural peculiarities. We conclude that the presence of various forms of sbmds in the PAG of senescent hamsters is a phenomenon that appears to be specific to the PAG region, but causal factors for this type of capillary degeneration remain unclear. Sbmds in the PAG may have serious consequences not only for blood–brain barrier functioning, but also for vascular perfusion and blood supply with eventually serious consequences for adequate regulation of the autonomic and motor control functions of the PAG region.

Key words: aging, capillary, blood–brain barrier, basement membrane, (micro)-vascular degeneration, spumiform basement membrane degeneration.

INTRODUCTION

Studies on aging and degenerative diseases related to compromised cognitive status mostly focused on cerebral cortical and hippocampal regions (Shah and Mooradian, 1997; De Jong et al., 1999; de la Torre, 2000, 2005, 2010a,b; Farkas and Luiten, 2001; Kalaria, 2003; Miller et al., 2007; Zlokovic, 2011). Despite the crucial role of the midbrain periaqueductal gray matter (PAG) as a key intermediary structure between higher order cortical regions and brainstem effector systems in the control of a myriad of autonomic and motor functions, there are hardly data available of aging effects on microvascular conditions in regions like the PAG.

The PAG is located in the gray matter of the mesencephalon surrounding the cerebral aqueduct and this position provides unique opportunities for connecting higher brain centers and brainstem effector

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Abbreviations: 3, ocular motor nucleus; BBB, blood–brain barrier; bm, basement membrane; CSF, cerebrospinal fluid; g, gliosis; ISF, interstitial fluid; PAG, periaqueductal gray; sbm, split basement membrane; sbmd, spumiform basement membrane degeneration.

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systems. Cytoarchitectonically, the PAG is not a homogeneous structure and already in 1954, the human PAG was divided into dorsal, medial and ventral subregions (Olszewski and Baxter, 1954). More recently, the cyto- and myeloarchitecture of the PAG has been described and partitioned in more detail (Liu and Hamilton 1980; Beitz, 1985; Beitz and Shepard, 1985; Veening et al., 1991; Gerrits et al., 1993) The PAG has been included in the concept of the 'greater limbic system' (Nieuwenhuys et al., 1988, 2008; Nieuwenhuys, 1996).

Functionally, the PAG is strongly involved in basic functions like the survival of the individual and the species, and plays a role in a variety of behavioral and physiological functions like: aggressive and defensive behaviors (Bandler and Depaulis, 1991; Bandler et al., 1991; Bandler and Shipley, 1994; Bandler and Keay, 1996), pain and analgesia (Giesler and Liebeskind, 1976; Mayer and Price, 1976; Basbaum and Fields, 1978; Fardin et al., 1984a,b; Besson et al., 1991), cardiovascular control mechanisms (Carrive, 1989; Carrive et al., 1989; Bandler et al., 1991; Bandler and Keay, 1996; Lovick, 1996), lordosis (Sakuma and Pfaff, 1979a,b, 1980, 1983) and estrous-cycle-related changes in neuronal responsiveness (Lovick et al., 2005; Lovick, 2006, 2008), and the vocal expression of emotions (Kanai and Wang, 1962; Jurgens and Pratt, 1979; Larson, 1985; Bandler et al., 1991; Jurgens, 1994). This wide-ranging functional involvement makes the PAG an important object to study the occurrence and effects of the vascular condition during aging, since all neural activity is directly dependent on adequate and effective blood supply.

The PAG is located around about the narrowest part of the ventricular system, the cerebral aqueduct (of Sylvius). Because of that special location around a narrow channel, the PAG is subjected to appreciable pressure changes induced by the pulsatile flow of the cerebrospinal fluid (CSF) (Stoquart-ElSankari et al., 2007; Klarica et al., 2009; Bulat and Klarica, 2011). For that reason, we decided to focus our attention on the effects of aging on microvascular integrity and condition in the PAG. Apart from microvascular age-associated changes we unexpectedly discovered capillaries with a new kind of vascular aberration, hitherto not previously reported (Gerrits et al., 2010, 2012b; Veening et al., 2012). Because of the 'foamy' electron lucent character of this particular aberration, we have termed these structures as 'spumiform basement membrane degeneration', (sbmd). In addition, we reported evidence for similar processes in rat and man (Gerrits et al., 2012b). Sbmd in the hamster PAG shares some characteristics with the membranous inclusions observed in the bm in the cerebral cortex (de Jong et al., 1990), and in the dorsal lateral geniculate nucleus of the aged rat brain (Alba et al., 2004), suggesting inter-species similarities. These findings open new ways for studies on capillary bm integrity in rat and mouse and man.

In the present study we performed a quantitative electron microscopical analysis comparing the sbmd aberrations in the rostral, intermediate and caudal PAG-microvasculature in young and aged hamster. We have chosen for this species because of our extensive previous findings on the relationship between the anatomical characteristics of the PAG and its autonomic functions in this mammalian species.

EXPERIMENTAL PROCEDURES

Animals

The experiments were performed on inbred animals obtained from Harlan (strain HsdHan: Aura; Harlan, Boxmeer, The Netherlands). Young (22 weeks, 120–122 g, cases H547, H548, H552, H556) and aged (95 ± 0.5 weeks, 130–140 g, cases H571, H574, H575, and H576) female golden hamsters (*Mesocricetus auratus*) were used for the present study. All protocols, concerning housing and handling of the animals and efforts to minimize animal suffering were in accordance with Dutch legalization and the ethical guidelines approved by the University of Groningen/University Medical Center Groningen (license number DEC 5142A).

Housing and handling

All hamsters were housed separately in clear plastic cages in a 14/10-h reversed light/dark cycle with food and water available *ad libitum*. Room temperature was maintained at 22–24 °C and humidity at 50–70%; wood shaving and straw were used as bedding materials. The animals were under daily monitoring for their general health condition and weighed once a week. Lifespan of hamsters varies considerably from 82–118 weeks depending on sex, strain and housing conditions (Kamino et al., 2001; Oklejewicz and Daan, 2002). Therefore, it was decided to euthanize aging animals at the age of 95–96 weeks, actually at the end of the female hamster lifespan.

Tissue processing

Perfusion. After an overdose of Nembutal (sodium pentobarbital, 50 mg/kg, i.p.; Lundbeck Inc., Deerfield, IL, USA), the animals were transcardially perfused with 20 mL of heparinized phosphate buffer (0.1 M, pH 7.4), containing 0.4% sodium nitrite and 2% polyvinylpyrrolidone (molecular weight 40,000) at 37 °C, followed by 350 mL of fixative containing 0.05% glutaraldehyde, 4% paraformaldehyde, 0.2% picric acid and 2% polyvinyl-pyrrolidone in 0.1 M phosphate buffer, pH 7.4, at room temperature. Following perfusion, the brains were removed and postfixed for one hour in the same fixative at 4 °C.

Electron microscopy. PAG tissue was cut on a vibratome into 60-μm transverse sections and collected in 0.01 M phosphate-buffered saline (PBS) at 4 °C. Every other section was processed for a standard EM protocol: osmicated, dehydrated in a graded series of ethanol and flat-embedded in Epon between dimethyldichlorosilane-coated glass slides. Samples of tissue containing the PAG and brainstem control regions were glued on Epon stubs. After blocking, the tissue was trimmed and cut into 1-μm semithin sections. Finally, 60-nm ultrathin sections from the selected regions were cut with a diamond knife for further electron microscopical analysis.