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Spumiform capillary basement membrane swelling: a new type of microvascular degeneration in senescent hamster

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ABSTRACT

Brain microvasculature plays a critical role in the regulation of homeostasis of neural tissues. The present study focuses on characteristic microvascular basement membrane (bm) aberrations in the midbrain periaqueductal gray matter (PAG) and their relation to aging. The PAG can be considered a caudal extension of the limbic system and is a key structure in the regulation of a myriad of autonomic and motor control functions. In an ultrastructural study, morphologic changes in mesencephalic PAG capillaries were assessed in aged and young hamster and compared with those in caudal brainstem areas. Bm aberrations were studied in 1200 capillaries ($n = 600$ young hamsters; $n = 600$ aged hamsters). A new, never reported variant of bm degeneration was found that presented itself as foamy-like structures accumulating within the lamina densa of notably PAG capillaries. We classified these foamy structures as 'spumiform basement membrane degenerations' (sbmd) in which we could distinguish 4 stages depending on the size and intramembranous localization, ranging from split bm (stage I), intermediate stages II and III, to extensive stage IV, affecting almost the complete capillary bm outline. In the PAG of senescent animals various stages of sbmd were observed in 92 ± 3% of all capillaries. Stage II was most prominently present (59%), followed by stage III (20%), and stage IV (13%). These bm aberrations were clearly age-dependent because in young animals, only 5% of the PAG capillaries showed characteristics of sbmd. For comparison, in the pontine reticular formation at the PAG-level, 41% of the capillaries showed a form of sbmd, but these defects were significantly less severe (stages I–II, 98%), and caudal brainstem structures displayed no sbmd at all. In addition to sbmd, diffuse endothelial changes, disrupted tight junctions, thickening of the bm, pericyte degeneration, and gliosis were observed in PAG capillaries. It is hypothesized that selective bm permeability of PAG capillaries results in a sequence of bm damage events that start with split bm, gradually changing into more and more extensive sbmd accumulations that eventually almost completely surround the capillary. Progressive sbmd in PAG capillaries might lead to a loss of blood–brain barrier function and consequently to impairment of autonomic and motor control functions exerted by the PAG.

1. Introduction

Most studies on aging of the vascular and microvascular condition in the mammalian brain focused on cerebral cortical and hippocampal regions, often in relation to neurodegenerative diseases and a compromised cognitive status (De Jong et al., 1999; de la Torre and Aliev, 2005; de la Torre; 2000, 2010a,b; Farkas and Luiten, 2001; Kalaria, 2003; Miller et al., 2007; Shah and Mooradian, 1997; Zlokovic, 2011). To our knowledge, no specific data are available from aging studies on microvascular conditions in subcortical regions such as the midbrain periaqueductal gray matter (PAG), despite the crucial role of the PAG in the control of a myriad of autonomic and motor control functions such as: control and expression of pain, analgesia, fear, anxiety, vocalization, lordosis, and cardiovascular function (Behbehani, 1995; Linnman et al., 2012; Paxinos and Mai, 2004).

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Exploring the neural substrate serving reproduction, we recently demonstrated a prominent columnar organization of nuclear estrogen receptor alpha (ER-α) immunoreactive neurons in the PAG projecting to the caudal brainstem of the female golden hamster, including nucleus retroambiguus, nucleus pararetroambiguus (NPRA) and commissural nucleus of the solitary tract (NTScom) (Gerrits et al., 2009b). The NPRA and the NTScom are part of a brainstem circuit comprising several interrelated nuclei that are subject to functional and structural plasticity and are intimately involved in the regulation of steroid hormone-dependent behaviors and their associated autonomic adaptations (Gerrits et al., 2008a,b, 2009b, 2010, 2012b).

As part of the ultrastructural study of steroid hormone responsive midbrain regions we included analysis of the microvascular condition in these steroid sensitive brainstem centers. No differences were observed between the microvascular changes occurring in the estrogen-receptive versus the estrogen-nonreceptive caudal brain stem areas of the female hamster brain. Despite commonly reported aging-associated neural and cerebrovascular degenerative changes including blood–brain barrier (BBB) impairment (Gerrits et al., 2010, 2012a), the animals displayed a reproductive behavioral repertoire comparable with young animals (Gerrits et al., 2009a; Veening et al., 2009). Furthermore, it was noticed that vascular degenerative aberrations like perivascular fibrosis which are commonly reported in the hippocampus of aging rats and other vertebrate species (De Jong et al., 1990; Farkas et al., 2001) were not seen in the brainstem of the aging hamster (Veening et al., 2009). Apparently, vascular impairments might be region-specific and not related to inhibitory components of estrous cycle-related behaviors.

Based on these and other observations we extended our interest to the aging effects on microvascular condition and structure in the midbrain PAG in view of the cardinal role of this brain region in autonomic regulation and its vulnerability during the aging process. Therefore, we analyzed the ultrastructure of the capillaries in the PAG in young (23 weeks) and aged (95 weeks) female hamsters and compared the PAG findings with estrogen-sensitive NPRA and NTScom, and the non–estrogen-sensitive medial tegmental field (mtf).

We unexpectedly discovered in the PAG capillaries a new kind of vascular aberration, as far as we are aware hitherto not described in the literature. Looking back into our previous investigations, we have observed and shown this aberration occasionally (Gerrits et al., 2010), but in the microvessels of the PAG this aberration turned out to occur most frequently. Especially in the PAG, successive stages in the development of this 'new' aberration can be observed. Because of the 'foamy' electron lucent character of the aberration, we have coined it as 'spumiform basement membrane degeneration' (sbmd). The regional differentiation of the occurrence of sbmd in the mesencephalic PAG and other brainstem areas forms the main content of our present study.

2. Methods

2.1. Animals

Four aged (95 ± 0.5 weeks) female golden hamsters (*Mesocricetus auratus*; cases H571, H574, H575, H576) weighing 130–140 g and 4 young (23 ± 0.5 weeks) female control hamsters (cases H547, H548, H552, H556), weighing 120–126 g were used for the present study. The experiments were performed on inbred animals obtained from Harlan (strain HsdHan: Aura; Harlan, UK Ltd.). All protocols, housing, and handling of the animals were in accordance with the ethical guidelines approved by the University Medical Center Groningen, University of Groningen (license number DEC 5142A). All necessary efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Housing and handling

All hamsters were housed separately in clear plastic cages in a 14/10-hour reversed light/dark cycle with food and water available ad libitum. Room temperature was maintained at 22 °C–24 °C and humidity at 50%–70%; wood shaving and straw were used as bedding materials. The animals were inspected daily for their general health condition and weighed once a week. Senescent animals were kept until 95–96 weeks, actually at the end of the female hamster lifespan (Gerrits et al., 2010, 2012b).

2.3. Tissue processing

2.3.1. Perfusion

After an overdose of nembutal (0.7 mL of 6% sodium pentobarbital intraperitoneally; 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% polyvinylpyrrolidone in 0.1 M phosphate buffer, pH 7.4, at room temperature. After perfusion, the brains were removed and postfixed for 1 hour in the same fixative at 4 °C.

2.3.2. Electron microscopy

Caudal brainstem and PAG tissue was cut on a vibratome in 60 μm transverse sections and collected in 0.01 M phosphate-buffered saline at 4 °C. Every other section was processed for a standard electron microscopy 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 structures were cut with a diamond knife for further electron microscopic analysis. At the ultrastructural level, 3 caudal brainstem structures (NPRA, NTScom, and mtf) and PAG were studied in detail. All microvascular and surrounding profiles were photographed at magnification × 10,000–20,000 using a Philips CM 100 electron microscope (Philips, Eindhoven, The Netherlands).

2.4. Control tissue

Brain tissue (PAG, NPRA, NTScom, and mtf) obtained from the young animals was processed exactly in the same way as the aged animals and served to control for possible aberrations as a result of tissue processing, and as a comparison group for aging-associated basement membrane (bm) degeneration. Tissue obtained from pontine reticular formation (prf) at the level ventrolateral to caudal PAG served as internal animal control (Fig. 1).

2.5. Photomicrography

The location of PAG, prf, NPRA, NTScom, and mtf were determined using a Zeiss Axioplan light microscope (Carl Zeiss Benelux, Trapezium 300, Sliedrecht, The Netherlands) at magnification × 10 (Fig. 1). Representative sections were photographed using a Leica DC500 digital camera and a Leica DM4000B photomicroscope connected to a Leica Q550IW computer and QWIN software (Leica Microsystems, Rijswijk, The Netherlands). Drawings of the sections were made using Adobe Illustrator 8.0 software (Adobe Systems, Mountain View, CA, USA).