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The arcuate nucleus as a circumventricular organ in the mouse

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ABSTRACT

The present study searched for morphological correlates of the permeability of the ventromedial arcuate nucleus of the mouse to blood-borne proteins. First, we determined that highly permeable microvessels are detected in the ventromedial arcuate nucleus using a rat monoclonal antibody to a mouse-specific endothelial phenotype (clone MECA32) recently recognized as a marker of endothelial fenestral diaphragms and previously shown to label circumventricular organs. Second, in the mild conditions of tissue fixation mandatory for use of MECA32, we observed that after a rapid vascular flush with saline, endogenous immunoglobulins are especially retained in circumventricular organs and ventromedial arcuate nucleus. The ventromedial arcuate nucleus thus shares features in common with classical circumventricular organs.

Although there was some consensus about the hypothalamic ventromedial (vm) arcuate nucleus (ARC) being, like the adjacent neuroendocrine median eminence (ME), outside the blood–brain-barrier, and thus about its function as a circumventricular organ (CVO) sensing plasmatic solutes mediating peripheral feedback [1,13], anatomical evidence for highly permeable fenestrated microvessels within its parenchyma, a cardinal feature of CVOs [8], had remained moderate [6,17]. For this reason, we recently [5] prepared an antiserum to a marker of fenestrated microvessels, the integral protein PV1 (for plamalemmal vesicle associated protein 1) [18]. PV1 is a 50–60 kDa protein (depending on glycosylation) that in homodimers constitute the radial fibrils of the diaphragms of fenestrated endothelial cells [2,19]. Our antiserum to PV1 proved efficient for visualizing permeable microvessels, in a subependymal position in the vmARC, as well as, expectedly, in all CVOs, ME and pituitary, where we also detected expression of PV1 mRNA by in situ hybridization histochemistry [5]. However, our antiserum to PV1 appeared rat specific, and it remains important to extend these observations to other animal species for validation of the vmARC-CVO functional synonymy, and especially to the mouse that has become an important animal model. Following the discovery of PV1, it became clear that an early released rat monoclonal antiserum to a mouse-specific endothelial 50–60 kDa antigen (the clone MECA32) [9] was recognizing this fenestral diaphragm-forming protein, as in the adult mouse brain MECA32 exclusively labels CVOs' microvessels including the primary portal plexus in the ME [16]. Because the labeling by MECA32 in the ARC was not detailed [16], we re-examined this point in the present study. Furthermore, to validate that MECA32-positive microvessels in vmARC functionally serve to deliver plasma solutes to the nervous parenchyma, we studied the distribution of endogenous blood-borne proteins, namely immunoglobulins (IgGs), and confronted this to the presence of MECA32-positive microvessels in the forebrain. We reasoned that, if IgGs are seen to be diffusely distributed in the parenchymatous environment of CVOs after intravascular aldehyde fixation [4], in the present mild conditions of tissue fixation mandatory for use of MECA32, these soluble proteins may be retained only where most abundant, i.e., at their sites of facilitated penetration.

Normal 12–16-week-old male C57BL/6J mice (CERJ Janvier, Le Genest St-Isle, France) were housed with water and food ad libitum (lights on 0730 h–19 h30 h) and killed, in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). After an overdose of 20% chloral hydrate animals were either let through death ($n = 3$) or rapidly perfused transaortically with saline to flush out blood before decapitation, this to compare the extent of IgGs' retention in brain ($n = 10$). Briefly, brains were frozen and sections were cut on a cryostat at 12 μm, collected on gelatinized slides and post-fixed for 1 min

Abbreviations: CVO, circumventricular organ; IgGs, immunoglobulins; ME, median eminence; MECA32, rat monoclonal antiserum to a mouse endothelial cell antigen; PV1, plasmalemmal vesicle associated protein 1; vmARC, ventromedial arcuate nucleus.

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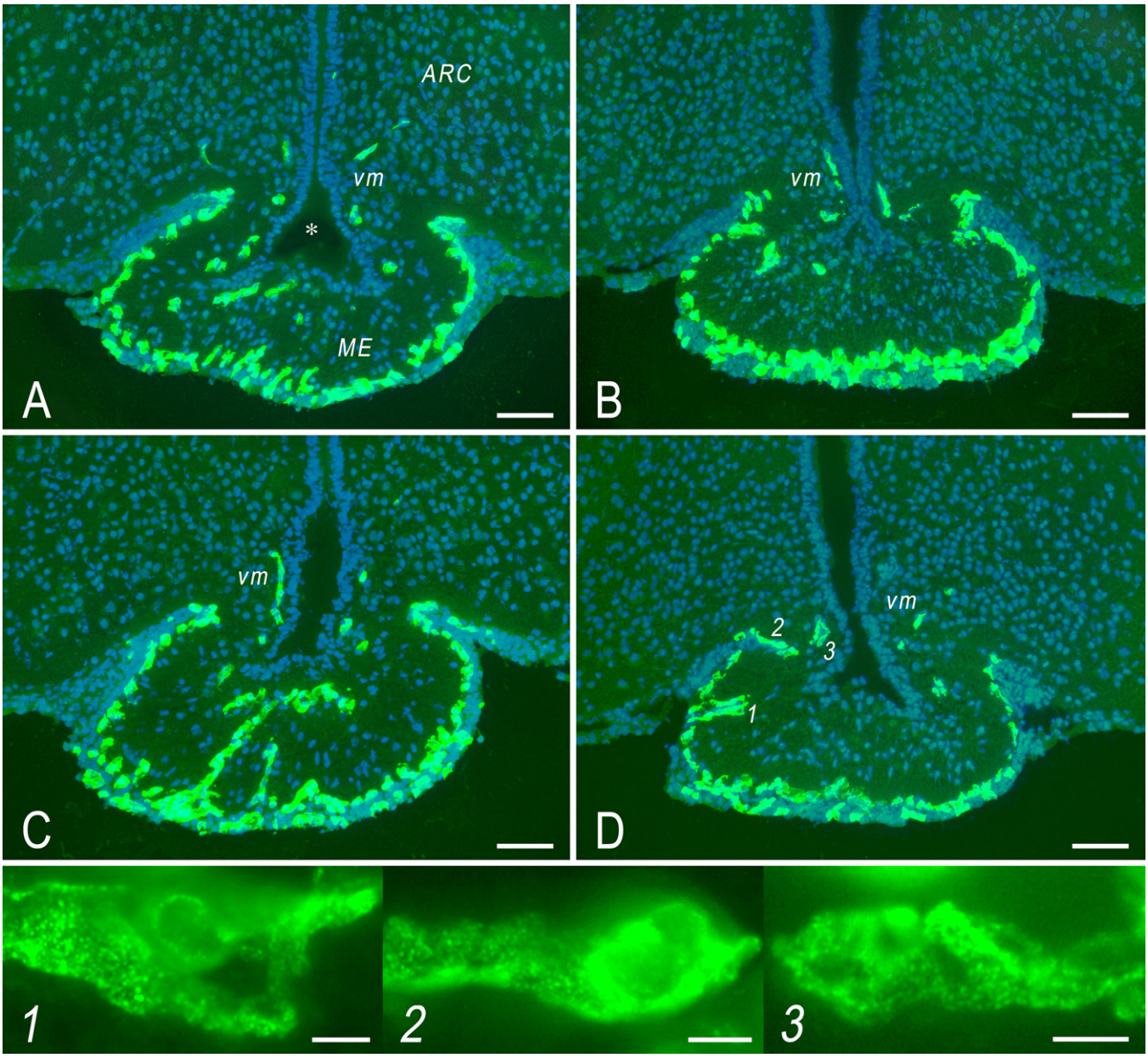

Fig. 1. Labelling by the permeable endothelium marker MECA32 (green) in frontal sections through the arcuate nucleus (ARC)–median eminence (ME) complex (A–D depict four different animals; blue Hoechst counterstain). Endothelial cells are detected both in the ME and ventromedial ARC (vm). Microvessels numbered in D are shown at a higher magnification in *1–3* through green filter only, labeling by MECA32 appears punctate. Bars = 100 μm in A–D and 5 μm in *1–3*. *, third ventricle.

in methanol/acetone (1 vol./1 vol.) at room temperature. Antisera were diluted in a 0.1 M veronal buffer, pH 7.4, containing 0.25% Triton-X100. First step incubation was overnight with rat clone MECA32 (1:750; BD Biosciences; Franklin Lakes, NJ, USA; Cat. No. 550563), a pan-endothelial marker rat anti-CD31 (1:300; clone MEC13.3; DB Bioscience; Cat. No. 550274) or donkey anti-mouse IgGs (linked to TRICT or Dylight 488; 1:500; Jackson Immunoresearch Labs; West Grove, PA, USA). Secondary step incubation was for 2 h (with donkey anti-rat and anti-mouse IgGs diluted 1:300). Observation was on Epifluorescence (Zeiss Axiophot 1 equipped with a Leica DFC300X video camera) and confocal (Leica DMR TCS SP2 AOBS) microscopes (Carl Zeiss S.A.S., Le Pecq, France; Leica Microsystems France, Rueil-Malmaison, France). See [5] for detailed procedures.

MECA32 distinctly generated punctate labelling (fenestral diaphragms) in cells of the vmARC (Fig. 1) that were positive for the panendothelial marker CD31. These MECA32-positive microvessels were clearly part of the sub-ependymal plexus and were most abundant in the portion of the vmARC closest to the pituitary stalk, a topographical arrangement that we established in the rat [5] where we identified diaphragmed fenestrated endothelial cells with electron microscopy [6]. The present findings confirm and extend previous observations of MECA32-positive microvessels in the CVOs and ME [12,16] and reflects a remarkable stability in angioarchitectural organization across animal species [1,7,13], suggesting that this permeable microvasculature of the vmARC is a specific feature of the mediobasal hypothalamus.

In the non-perfused brains, labelling for IgGs was profuse in all blood vessels including MECA32-positive microvessels in CVOs (organum vasculosum laminae terminalis, subfornical organ) and choroid plexi (Fig. 2A–C), as well as in ARC and ME (Fig. 3A). In the rapidly perfused brains, labelling for mouse IgGs was essentially apparent around microvessels – in the CVOs (Fig. 2D–G), choroid plexi (Fig. 2H and I), ME and ARC (Fig. 3B–G) – that