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Role of capillary pericytes and precapillary arterioles in the vascular mechanism of betahistine in a guinea pig inner ear model

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ABSTRACT

Aims: Betahistine is a histamine analogue that is used for the treatment of Menière's disease. Animal studies showed that it increases local blood flow in the stria vascularis. In terms of its mode of action, recent studies have prompted discussion of whether betahistine actively affects cochlear microcirculation by dilations of pericytes or of precapillary arterioles or by mere downstream effects. Hence, we investigated the effects of betahistine on cochlear capillary pericytes and precapillary arterioles.

Main methods: The stria vascularis was visualized in 12 guinea pigs by in vivo fluorescence microscopy. In these, 152 pericytes were stained and local diameter at sites of pericyte somas and downstream controls as well as intravascular blood flow were measured before and after betahistine application. Moreover, in two guinea pigs the precapillary arterioles were visualized by 2-photon-microscopy before and after betahistine application.

Key findings: There was no significant change in capillary diameter at sites of pericyte somas after betahistine application compared to controls, baseline or downstream controls, even though cochlear blood flow increased significantly. The two-photon measurements indicated an active dilation of precapillary arterioles.

Significance: Since we found no evidence that betahistine affects cochlear microcirculation by cochlear pericytes, its main mode of action is evidently active dilation of pre-capillary arterioles. These findings are in line with similar effects reported in the central nervous system and indicate an active effect on cochlear microcirculation.

1. Introduction

Menière's disease is clinically characterized by recurrent attacks of vertigo lasting minutes to hours, impaired hearing, tinnitus and fullness in the affected ear and was recently reclassified [1]. It is most likely caused by an endolymphatic hydrops [2,3], in which ruptures of the physical barriers of the endolymphatic space cause the recurring attacks of the aforementioned symptoms. Menière's disease is the second most common cause of otogenic vertigo [4].

Treatment options vary from dietary restrictions [5] to oral diuretics [6], intratympanic application of dexamethasone [7], lidocaine [8] or gentamycin [9]. In otherwise untreatable cases, surgery of the vestibular organ may be considered, even though the outcome is uncertain and associated with severe side effects [10]. Oral administration of betahistine hydrochloride, a histamine analogue, is a common treatment, in particular in central Europe [11]. It is generally considered beneficial in reducing the episodes of vertigo that are associated with Menière's disease, [11] although a randomized-controlled trial showed no benefit in the daily dosages examined [12].

Two modes of action have been proposed for the use of betahistine in vertigo and dizziness: Firstly, the inverse agonism of betahistine at the histaminergic H_3-receptor [13,14] is believed to aid the central nervous compensation in the vestibular nuclei in the case of a peripheral vestibular imbalance [15]. The second mode of action proposed is an active increase of cochlear microcirculation in the stria vascularis, [16–19] also mediated by its inverse agonism at the H_3-receptor and its interaction with the adrenergic α_2-receptor [14]. This is believed to lead to a reduction of the endolymphatic hydrops.

Studies dealing with the cochlear effects of betahistine have also shown systemic circulatory effects that match the changes in cochlear

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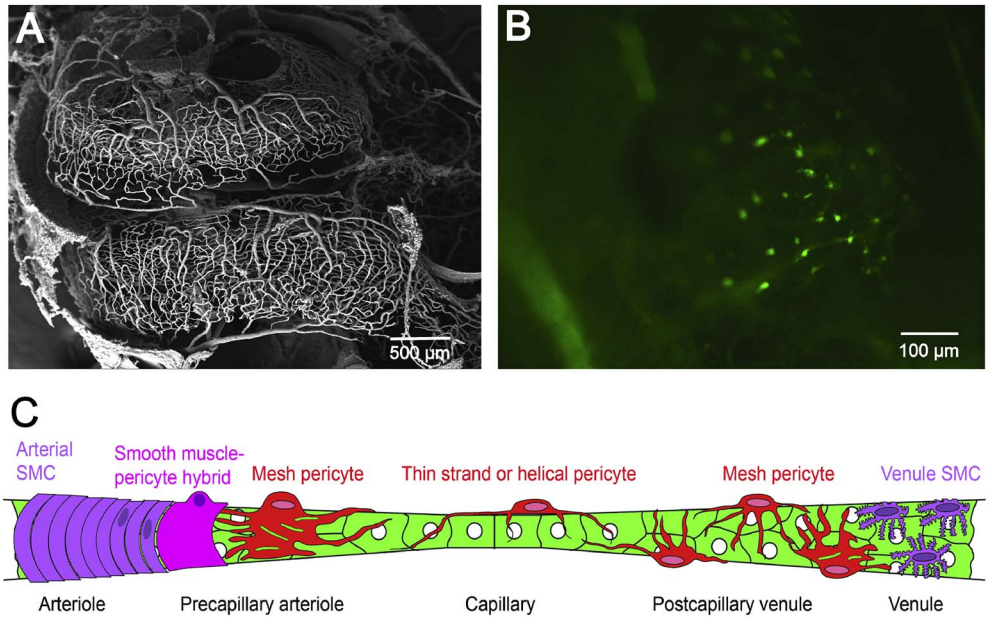

Fig. 1. A) Schematic display for pericyte heterogeneity, reproduced with permission from Hartmann et al. [45] B) Cast of the cochlear vascular network, reproduced with permission from Carraro et al. [46] C) In-vivo fluorescence microscopy of the stria vascularis with stained pericytes in a guinea pig.

microcirculation [14,16,18,19]. Hence, these observations gave rise to the question whether the increases in cochlear microcirculation are the results of a) an active regulation of cochlear blood flow or b) mere downstream effects.

In line with the argument of an active increase of cochlear blood flow is the fact that studies dealing with local regulation of blood flow in the cerebral cortex showed that the cerebral capillary pericytes take an active role in the regulation of capillary blood flow [20]. Pericytes are a heterogenous group of cells that adhere to the outer wall of capillaries (Fig. 1A, C). They play an important role in capillary stabilisation and regulation of local blood flow [21] and also seem to play an integral role in numerous central nervous diseases [22]. Since the capillary pericytes of the stria vascularis exhibit similar functional properties as the central nervous pericytes, such as the ability to contract and relax [23], and are of a similar embryological origin, a similar function of the capillary pericytes is probable. This view is further supported by the fact that exposition to tumor necrosis factor, a mediator in numerous inner ear pathologies, is capable of inducing active decreases in capillary diameters at sites of pericyte somas, while neutralisation of tumor necrosis factor causes a return to basal values [47]. A potential mode of action for betahistine is a direct effect on capillary pericytes, since pericytes have been known to increase local microcirculation upon H_1-stimulation in the retina [24] as well as expressing adrenergic receptors [25]. Additionally, some authors even consider the pre-capillary arterioles (Fig. 1A) to be a subpopulation of pericytes, [26,27] structures that have also been postulated to play a role in local regulation of cochlear blood flow [28].

In the light of these findings, we propose that if the effects of betahistine on cochlear microcirculation are specific to the cochlea, they will either be mediated by an active effect of betahistine on cochlear capillary pericytes, including the pre-capillary arterioles. Therefore, we investigated the effect of betahistine on a) cochlear capillary pericytes and b) pre-capillary arterioles.

2. Materials and methods

2.1. Ethics statement

All of the experiments in this study were reported to and approved by the responsible animal protection authorities ("Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit, Oldenburg", Germany) under the license number 33.9-42502-04-12/0889.

2.2. Animals

The guinea pig is a well-established animal model for microcirculation [29] as well as Menière's disease [30]. The laboratory animals were guinea pigs from the Dunkin-Hartley strain bought directly from approved retailers (Envigo RMS GmbH, Rossdorf, Germany) weighing from 200 g to 450 g. Anesthesia was induced by intramuscular injection of a combination of ketamine (50 mg/kg bodyweight) and xylazine (5 mg/kg bodyweight) and sustained by repeated injections of half the dosage every 30 min.

2.3. Surgical approach

The surgical approach in order to visualize capillary cochlear microcirculation and capillary pericytes used in this experiment has been described repeatedly by this group [31–33] as well as others [34]. Thirty minutes prior to induction of anesthesia, an intraperitoneal dose of buprenorphine (0.5 mg/kg bodyweight) was given. After induction of anesthesia by the above mentioned protocol, the hair overlying the left lateral cervical region as well as the right periauricular area was removed. Following this, local anesthesia (lidocaine with epinephrine) was applied intracutaneously. Initially, an intravenous catheter was inserted into the left jugular vein, allowing i.v.-application of fluids as well as contrast material. After the implantation, the right external ear was removed and the bulla containing the cochlea was mechanically opened. After this, the periosteal vessels present in the bulla were removed above the cochlea. Then, an incision into the bony shell of the cochlea was carved above the second turn, using a no. 11 scalpel. The window carved measured approximately 500 × 500 µm. After the operation site had been rinsed with sterile saline solution, contrast material (fluorescein-labelled dextrane, molecular weight 500,000; 0.05–0.1 ml of a 5% solution in 0.9% NaCl; Sigma-Aldrich, Deisenhofen, Germany) was applied intravenously. In-vivo microscopy was then done by direct illumination with a Leica EL6000 light source (Leica Microsystems, Wetzlar, Germany) connected to a Leica M205 FA stereomicroscope (Leica Microsystems, Wetzlar, Germany). Once a portion of the stria vascularis had been visualized, pericytes were stained by topical application of a 5 mM of 4,5-diamofluorescein diacetate in dimethyl sulfoxide solution (Sigma-Aldrich, Deisenhofen, Germany) diluted 1:10 with sterile saline for 20 min. The images