Auris Nasus Larynx 41 (2014) 249–254

Contents lists available at ScienceDirect

Auris Nasus Larynx

journal homepage: www.elsevier.com/locate/anl

Effects of arginine vasopressin on auditory brainstem response and cochlear morphology in rats

Hideaki Naganuma [a,*], Katsumasa Kawahara [b], Koji Tokumasu [a], Ryohei Satoh [b], Makito Okamoto [a]

[a] Department of Otorhinolaryngology, Kitasato University School of Medicine, 1-15-1 Kitasato, Minami-ku, Sagamihara, Kanagawa 252-0374, Japan
[b] Department of Physiology, Kitasato University School of Medicine, 1-15-1 Kitasato, Minami-ku, Sagamihara, Kanagawa 252-0374, Japan

ARTICLE INFO

Article history:
Received 23 June 2013
Received in revised form 1 December 2013
Accepted 9 December 2013
Available online 7 January 2014

Keywords:
Meniere's disease
Endolymphatic hydrops
Arginine vasopressin
Intermediate cell
Stria vascularis
Vacuole

ABSTRACT

Objective: This study was conducted to evaluate the relationship between hearing and cochlear histopathology after arginine vasopressin administration in rats.
Methods: A total of 30 Wistar rats were injected with either 0.02 unit/g of arginine vasopressin or the same amount of isotonic saline solution. The initial auditory brain stem response threshold was recorded and additional measurements were made at 10, 30, 60, and 90 min after injection of arginine vasopressin or isotonic saline solution. The threshold for each timepoint was compared with the initial threshold. Histological quantitative assessment of endolymphatic hydrops in the cochlea was performed using light microscopy and assessment of the basal, intermediate, and marginal cells of the stria vascularis was performed with electron microscopy.
Results: The auditory brain stem threshold 60 min after arginine vasopressin injection increased significantly in comparison with the initial threshold ($P < 0.05$). Although the index for endolymphatic hydrops in rats administered arginine vasopressin was not different from that in controls ($P > 0.05$), vacuoles in the intermediate cells were increased significantly in the treated rats ($P < 0.01$).
Conclusion: Hearing impairment was detected without endolymphatic hydrops in rats administered arginine vasopressin. An increase of vacuoles in the intermediate cells may account for the hearing impairment induced by arginine vasopressin injection.

Introduction

Endolymphatic hydrops, a histopathological finding in the inner ear of patients with Meniere's disease, is caused by morphological changes in the labyrinth brought about by an imbalance of water and ion metabolism. This disorder is characterized by a bulge in Reissner's membrane due to enlargement of the scala media space [1,2]. The mechanisms responsible for the development of this condition remained to be clearly understood.

A number of clinical and experimental studies have suggested that arginine vasopressin (AVP) may be involved in the development of endolymphatic hydrops [3–9]. Researchers have reported that administration of a large amount of AVP can induce endolymphatic hydrops in guinea pigs [5,7]. These studies revealed dilatation of the scala media associated with extension of Reissner's membrane toward the scala vestibuli in histological sections. It is well known that secretion of intrinsic AVP increases with high serum osmotic pressure and/or with a decrease in circulatory blood volume to prevent loss of water and mineral ions from the body. Physical or mental stress also becomes a factor involved in increased secretion of intrinsic AVP [10].

In previous reports detailing the development of endolymphatic hydrops in guinea pigs, the question remained as to whether or not AVP administration could lead to hearing impairment. Thus, our study was planned to explore the relationship between hearing and both light and electron microscopic findings in the inner ear after AVP administration in rats.

Materials and methods

This study was approved by Kitasato University School of Medicine, Animal Care and Use Committee. A total of 30 male 5-week-old Wistar rats weighing 100–200 g were used. After administration of AVP or isotonic saline solution the following three evaluations were performed: (1) recordings of auditory brainstem responses (ABR) to reveal hearing impairments, (2)

* Corresponding author. Tel.: +81 42 778 8432; fax: +81 42 778 8441.
E-mail addresses: naganuma@kitasato-u.ac.jp (H. Naganuma), kawahara@nc.kitasato-u.ac.jp (K. Kawahara), cjb14540@ams.odn.ne.jp (K. Tokumasu), ryou@kitasato-u.ac.jp (R. Satoh), okamotom@med.kitasato-u.ac.jp (M. Okamoto).

0385-8146/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.anl.2013.12.004

histological study of the inner ear by light microscopy to evaluate development of endolymphatic hydrops, and (3) electron microscopy (EM) of the stria vascularis to quantify the cytoplasmic vacuoles. Both side of ear in each rat were used for the evaluations.

Hearing impairment evaluation

The initial ABR threshold was recorded, after which 0.02 unit/g of AVP (Pitressin; Arg-vasopressin, Daiichi-Sankyo, Japan) was injected intraperitoneally to five rats and the same volume of isotonic saline solution injected to five control rats. The ABR threshold was measured at 10, 30, 60, and 90 min after the injection. The interval time between the two latencies of the first and third peak-wave of ABR was measured at each timepoint.

Measurements were made in a soundproof room. Rats were anesthetized with intraperitoneal pentobarbital sodium (35 mg/kg). Two silver-coated screws, 1 mm in diameter, were inserted on the midline of the vertex (one on the forehead as a negative and the other as a positive electrode). A ground electrode was inserted subcutaneously into the back. Click sounds of 0.1-ms duration were given for each ear through a speculum connected to a headphone at a rate of 10 Hz. A total of 512 responses were band-pass filtered at 50–3000 Hz and averaged with a Neuropack Sigma system (Nihon Koden, Tokyo, Japan).

ABR thresholds were determined by visual detection of reproducible responses, with a descending series of click intensities in 10-dB sound pressure level (SPL) steps. We regarded a reproducible response observed within 10 ms after a click presentation as positive, and the smallest intensity of clicks that evoked a visually detectable response of the first peak as the threshold. All responses were double traced to confirm their reliability.

Histological study of the inner ear

AVP at a dose of 0.02 unit/g or the same volume of isotonic saline was administered to rats in AVP group (five rats) or control group (five rats), respectively. One hour after the injection rats were transcardially perfused with isotonic saline while deeply anesthetized with pentobarbital sodium. Fixation was performed with 4% paraformaldehyde. The temporal bone was removed and further fixed in 4% paraformaldehyde in a cold room at 4 °C for $\geq$10 days. Thereafter, specimens were decalcified with 10% ethylenediaminetetraacetic acid (EDTA) for three weeks, dehydrated in a graded ethanol series, and embedded in Technovit 7100 (Heraeus Kulzer GmbH & Co. KG, Wehrheim, Germany). Blocks were cut horizontally into 10-μm sections, stained with hematoxylin and eosin, and observed under a light microscope (Olympus Provis AX80, Tokyo, Japan).

For quantitative assessment of morphological changes in the endolymphatic space, ratios of the length (Ir-L) of Reissner's membrane and a cross-sectional area of the scala media (Ir-S) were measured from the mid-modiolar section of the cochlea. The measurements and evaluation were performed as previously described [6], with some improvements. The temporal bones were decalcified with 10% EDTA instead of 5% trichloroacetic acid, and embedded in Technovit 7100 instead of paraffin–celloidin mixture. These changes may have improved the evaluation of the Ir-L and Ir-S ratio because the shrinkage ratio of tissues using the new agents was smaller than that previously reported. Ashlar-Vellum 3D (Ashlar, Austin, TX, USA) measuring software was used for the analysis and both ears from each rat were assessed.

EM of the stria vascularis

One hour after the injection of 0.02 unit/g of AVP or the same volume of isotonic saline solution, five rats from each group were euthanized and their temporal bones removed. Two small holes were made on the lateral bony wall, one on the cochlea area of the apical turn and the other on the vestibular organ. The cochleae in the bilateral inner ears were then fixed by perilymphatic perfusion of fixative containing 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) via the hole on the wall of the vestibular organ. The precise time between euthanasia and tissue fixation was measured to ensure fixation quality and enable reliable comparisons.

Each extirpated cochlea was immersed in the same fixative for 2 h at 4 °C. The tissue samples were washed in 7% sucrose in 0.1 M phosphate buffer (pH 7.2) at 4 °C overnight. Then, they decalcified with 10% EDTA for 5 weeks, trimmed at the columella cochlea plane, and fixed with 2% buffered osmium tetroxide for 2 h. After fixation, the tissue was washed in 0.1 M phosphate buffer (pH 7.2), dehydrated in a graded ethanol series, and embedded in Quetol 651 resin mixture (Nissin EM Co., Tokyo, Japan). Ultrathin sections were stained with 3.5% uranyl acetate for 25 min and lead citrate for 5 min, and then observed under transmission EM (TEM; JEM-1230; JEOL Ltd., Tokyo, Japan) at 80 kV.

The stria vascularis facing the tympanic cavity at the second turn in the mid-modiolar section of the cochlea was selected for assessment because the second turn was the most stable during histological section preparation, because the apical and basal turns may have been damaged by the holes made for perilymphatic perfusion. For qualitative analysis, the whole stria vascularis was observed. As there was enlargement of an area lacking intracellular organelles in the intermediate cells or a so called "vacuole" was observed, it was quantitatively assessed. In the intermediate cells, vacuoles of various sizes existed. Microvacuoles with a diameter $<$1 μm were indistinguishable from the normal space between the intracellular organelles. Therefore, in the present study, "vacuole" was defined as a nonstructural area with a diameter $\geq$1 μm. Attempts were made to quantitatively evaluate the difference in the total amount of defined "vacuoles" in the intermediate cells in the whole of the stria vascularis between the control and AVP groups. The stria vascularis was photographed at 2000× magnification using TEM, scanned at 400 dpi, and saved in a computer. The images were then merged with Adobe Photoshop Element 3.0 software (Adobe Systems Incorporated, Mountain View, CA, USA) and the entire stria vascularis saved as one image (Fig. 3A and B). The total area of each defined "vacuole" in the intermediate cells was measured in the whole of the stria vascularis with National Institute of Health (NIH) Image J ver. 1.44 software (http://rsb.info.nih.gov/ij/index.html).

To account for size disparity of each stria vascularis, the total area of defined "vacuoles" and the stria vascularis were measured in the same way and the ratios of the former to the latter calculated and compared between the control and AVP groups. The portion of the stria vascularis hidden behind the bar of the grid mesh (EM fine grid, F-150 mesh, Nissin EM Co.) for TEM was excluded from the calculations (Fig. 3A). The absence of defined "vacuoles" in marginal and basal cells was also confirmed.

Statistical analyses

A Mann–Whitney *U*-test or Wilcoxon signed-rank test was applied to find the difference between two independent or paired groups. To detect significance among ABR thresholds of a series of timepoints, a Friedman test was used to obtain a global *P* value, and a Steel test was used to obtain pair-wise significance by comparing each ABR value to the initial ABR. A *P*-value $\leq$0.05 was considered statistically significant. All reported *P*-values are two-sided. Analyses were performed using GraphPad Prism version 4 (GraphPad Software, Inc., San Diego, CA, USA), and SPSS version 19.0 software (SPSS Inc., Chicago, IL, USA).