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Acupuncture Research

Arginine Vasopressin-Aquaporin-2 Pathway-Mediated Dehydration Effects of Electroacupuncture in Guinea Pig Model of AVP-Induced Endolymphatic Hydrops*

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ABSTRACT **Objective:** To investigate the effects of electroacupuncture (EA) on endolymphatic hydrops (EH) and the regulation of arginine vasopressin (AVP)-aquaporin-2 (AQP2) pathway in guinea pigs. **Methods:** EH was induced in male guinea pigs by an intraperitoneal injection of AVP. For the treatment, EA was delivered to Baihui (GV 20) and Tinggong (SI 19) acupoints, once per day for 10 consecutive days. In histomorphological studies, cochlear hydrops degree was evaluated by hematoxylin-eosin (HE) staining, and then the ratio of scala media (SM) area to SM + scala vestibuli (SV) area (R value) was calculated. In mechanical studies, a comparison of plasma AVP (p-AVP) concentrations, cyclic adenosine monophosphate (cAMP) levels, vasopressin type 2 receptor (V2R) and AQP2 mRNA expressions in the cochlea were compared among groups. **Results:** EA significantly reduced cochlear hydrops in guinea pigs (P=0.001). EA significantly attenuated the AVP-induced up-regulation of p-AVP concentrations (P=0.006), cochlear cAMP levels (P=0.003) and AQP2 mRNA expression (P=0.016), and up-regulated the expression of V2R mRNA (P=0.004) in the cochlea. **Conclusion:** The dehydrating effect of EA might be associated with its inhibition of AVP-AQP2 pathway activation.

KEYWORDS endolymphatic hydrops, electroacupuncture, arginine vasopressin (AVP)-aquaporin-2 (AQP2) pathway, Meniere's disease

Meniere's disease is a disorder of the inner ear that affects a large number of patients. Endolymphatic hydrops (EH) is generally considered to be a major pathological basis in case of Meniere's disease.[1] The underlying mechanisms that lead to overaccumulation of endolymph are not known so far. However, there is considerable clinical and experimental evidence for the belief that EH in the inner ear is in part resulted from the mal-regulation of the arginine vasopressin (AVP)-aquaporin-2 (AQP2) pathway.[2] According to Beitz, et al,[3] the regulation of the endolymph water homeostasis by AVP-AQP2 pathway in the inner ear may occur by similar molecular mechanisms as in the kidney. AVP should act on vasopressin type 2 receptor (V2R) and therefore increase the number of AQP2 via a cyclic adenosine monophosphate (cAMP)-mediated phosphorylation in the inner ear, thus greatly promoting water influx into the endolymphatic compartment.

Electroacupuncture (EA), a mean of electrically stimulating the points via acupuncture needles, has proven more repeatable and adjustable comparing with traditional acupuncture therapy.[4] Our previous animal experiments suggested that EA at selected acupoints [e.g., Baihui (GV 20) and Tinggong (SI 19)] can reduce the excess water retention in the endolymph and improve hearing function in guinea pigs with EH.[5,6] More importantly, a line of clinical evidence has also revealed the beneficial effects of EA, where it has excellent results in control of symptoms, primarily of episodic vertigo, but also in terms of hearing loss and tinnitus.[7] Despite these benefits of EA, the detailed molecular mechanisms of EA treatment for Meniere's disease still remain an enigma, and the influence of EA on AVP-AQP2

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pathway activation in the inner ear which is associated with EH is unclear. Systemic administration of AVP to a guinea pig provides a suitable model to understand the mechanism involved in EH.[8,9] Consequently, we used a guinea pig EH model to observe the dehydrating effects of EA and to examine whether EA may reduce hydrops by suppressing the activation of AVP-AQP2 pathway.

METHODS

Animals

For the experiments described here, a total of 52 healthy male albino guinea pigs with a positive Preyer's reflex and weighing 350–400 g were selected from the Animal Center of Zhejiang Chinese Medical University, Hangzhou, China [license No. SCXK (Zhejiang) 2013-0060]. All animals were acclimated for 7 days in cages in a quiet room immediately before the experiment procedure.

All experimental protocols were approved by Animal Care Committee of Zhejiang Chinese Medical University, Hangzhou, China. All experiments conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Experiment Design

In histomorphological studies, 10 guinea pigs were used to examine the model of EH after 7 days of AVP administration. Five animals without treatment served as control (group 1). The remaining 5 served as experimental animals (group 2). Based on successful establishment of EH model, the effects of EA on cochlear morphology of guinea pigs were evaluated, and animals were exposed to EH (group 3) or EA after EH (group 4, EA at points GV 20 and SI 19 on the left side, *n*=5 in each group). Left side of temporal bone was removed for quantitative analysis of the morphologic changes of the endolymphatic space in the above experiments.

In mechanical studies, the influence of EA on AVP-V2R-cAMP-AQP2 pathway was examined. Guinea pigs were randomly divided into 4 groups of normal ears (group 5), hydropic ears (group 6), hydrops + EA at points GV 20 and SI 19 on the left side (group 7) and hydrops + EA at points GV 20 and SI 19 on the right side (group 8, *n*=8 in each group). Blood samples were collected to measure plasma AVP (p-AVP) concentrations using radioimmunoassay (RIA) system. Left side of cochleae in groups 5, 6 and 7 were dissected out for the measurement of cAMP levels using RIA, and the right side of cochleae in groups 5, 6 and 8 were collected to measure the content of V2R and AQP2 mRNA expressions using quantitative real-time PCR (qRT-PCR).

Establishment of EH Model

In the present study, EH guinea pig model was induced by a once-daily intraperitoneal injection of [Arg]8 -VP (V9879, Sigma-Aldrich, Co., St. Louis, MO, USA) dissolved in distilled water at a dose of 4 μg·kg^{-1}·day^{-1} for successive 7 days.[9]

EA Stimulation

After model establishment, guinea pigs were gently restrained in a self-made fixing device with the head outside. EA treatment was delivered to GV 20 (in the midline of the head, approximately at the midpoint of the line connecting the apexes of the two auricles) and unilateral SI 19 (in the depression anterior to the tragus), as reported previously.[5,6] Briefly, Huatuo brand disposable needles (0.25 mm diameter with 25 mm in length, Suzhou Medical Apparatus Limit, Jiangsu Province, China) were inserted to GV 20 about 2 mm horizontally to the skin and to SI 19 about 3 mm vertically. Electrical stimulation (a current of 1 mA, a frequency of alternating disperse and dense wave, 2 Hz versus 15 Hz stimulation) was delivered using Han's acupoint nerve stimulator (HANS 100A, Nanjing Gensun Technology, Jiangsu Province, China) for 20 min. The control groups were all loosely immobilized for 20 min. All treatments were started at day 1 after EH was established, and repeated once per day for 10 days.

Fixation Procedure

Following treatment, animals were killed using a sublethal administration of chloral hydrate (10% w/v, 300 mg/kg intraperitoneally), and intracardiac perfusion was performed with 4% paraformaldehyde (pH=7.2). Afterwards, left side of temporal bones were dissected cautiously and small holes were created on the apex turn and round window of each cochlea respectively, then the cochleae were immersed in the fixative at room temperature overnight. After fixation, all cochleae were subjected to decalcification in 10% ehthylene diamine tetraacetic acid (EDTA) for approximately 3 weeks, dehydrated in a graded ethanol series, and then embedded in paraffin-celloidin mixture. Blocks were sectioned horizontally into 4.5 μm sections.

These sections were stained with hematoxylin and eosin (HE), and examined using a light microscope.

Degree of EH

The light microscopical examination of changes of endolymphatic space was performed by investigators in a blind fashion. The second turn in the mid-modiolar section of each cochlea was selected and images were captured with a digital camera (Nikon Cool PIX990, Tokyo, Japan). Here only the second turn was selected for assessment because of the good stability of the second turn during histological section preparation.[10] The area of the scala media (SM) and scala vestibuli (SV) for the second turn of the cochlea on both sides of the modiolus was measured as previously described.[11] There were two measurements in each cochlea of each animal. Then the ratio of SM area to SM + SV area (R value) was calculated. The hydrops degree of each animal was rated according to the average R value for the second turn on both sides of the modiolus.

Determination of p-AVP Concentrations

After therapeutic interventions, blood sample collections were carried out between 8:30 am and 10 am as a rule, while animals were anesthetized by the same method as mentioned above. The heart was exposed at the fourth intercostal space, and blood samples were collected from the heart into one heparin sodium anti-coagulation vacuum tube. The blood volume was not less than 2 mL. Blood samples were immediately centrifuged at 3,000 r/min for 15 min at 4 ℃, the supernatants were withdrawn and plasma samples were kept frozen at –80 ℃ until analyzed for AVP. Plasma levels of AVP were determined by a RIA, using the AVP-RIA kit (Lot No. 20150520; supplied by the Second Mililtary Medical University of People's Liberation Army, Shanghai, China). According to the manufacturer's protocol, one vial of lyophilized AVP standard was reconstituted with 2 mL of buffer solution supplied in the kit. From this solution (12.8 ng/mL, w/v), each AVP standard (2.5, 10, 40, 80, 160, 320, 640, 1280 pg/100 μL) was made by serial dilution with buffer solution. Then 100 μL AVP standards and 200 μL of buffer solution or 300 μL were mixed with 100 μL AVP antiserum. A volume of 300 μL plasma samples were mixed with 100 μL AVP antiserum. And then the solution in each tube was incubated together, at 4 ℃ for 24 h, allowing the antigen to bind with the antibody. Afterwards, 100 μL radiolabelled antigen ([125I] AVP) dissolved in assay buffer (pH=7.6) was added and the incubation was continued for 24 h at 4 ℃. And then 500 μL polyethylene glycol (PEG) solution was added, followed by vortexing and further incubation for 45 min at room temperature. The reaction tubes were centrifuged at 4,000 r/min for 20 min at room temperature, the resultant supernatant was aspirated and radioactivity in the pellet was determined using a gamma counter. Fitting of the standard curves and calculations were done with the aid of a computer. Concentrations of p-AVP were expressed as pg/μL.

Cochlear cAMP Assay

To measure the cAMP concentration, 1 mL HCL (1 mol/L) was added to finely frozen cochleae tissue in glass homogenizer, and samples were homogenized. The homogenates were transferred to 2 mL test tubes and incubated at room temperature for 100 min. Then the samples were centrifuged at 4,000 r/min for 20 min at 4 ℃ and the supernatants were collected. The supernatants were added to 0.8–1 mL NaOH to neutralize the HCL and then freeze-dried at –30 ℃ for 12 h. The cAMP content was determined by RIA, using cAMP RIA kit (Lot No. 20150427; Beijing Sino-UK institute of Biological Technology, Beijing, China) following the manufacturer's protocol. Briefly, each reaction system contained 100 μL of each sample, 5 μL acetylated reagent, 100 μL of anti-cAMP antibody and 100 μL of 125I-cAMP. Tubes were vortexed and incubated at 4 ℃ overnight. Then 100 μL normal rabbit serum and 100 μL of goat anti-rabbit IgG were added, the incubation was continued at 4 ℃ for overnight. And all tubes were centrifuged at 3,000 r/min for 15 min. Supernatants were discarded, the resulting sediments were counted for 1 min. By measuring the radioactivity of the precipitates, the cAMP content was calculated and presented in pmol/mL.

Gene Expression Analysis by qRT-PCR

V2R and AQP2 mRNA in the cochlea were measured using qRT-PCR with PrimeScript™ RT Master Mix (Perfect Real Time; Lot No. AK2602; Takara Biotechnology, Dalian, China) and LightCycler FastStart DNA Master SYBR Green I (Lot No. 11750100; Roche Applied Science, Mannheim, Germany) and expressed as $2^{-\triangle\triangle Ct}$. β-actin was used as an internal control. The primers for V2R were forward 5'-TCATCGTCTACGTGCTGTGC -3' and reverse 5'-TTGCAAGTAGCATGAGCAGC -3'. The primers for AQP2 were forward 5'-GCCCTCTCCATCGGTTTCTC-3' and reverse 5'-AGGATTCATGGAGCAGCCAG-3'.

The primers for β-actin were forward 5'-GTGGATCAGCAAGCAGGAGT-3' and reverse 5'-AGGGTGTAACGCAGCAAAGT-3'. The qRT-PCR was carried out in Chromo 4 real time PCR system (Bio-rad, USA). A standard cycle was applied for qRT-PCR reaction: an initial preinbubation period of 10 min at 95 ℃, followed by 40 cycles of denaturation for 10 s at 95 ℃, annealing at 60 ℃ for 20 s, and elongation for 15 s at 70 ℃. All samples analysis were performed in triplicates.

Statistical Analyses

All data was statistically analyzed using SPSS for windows (Version 19.0, SPSS Inc., Chicago, United States). The Kolmogorov–Smirnov test was performed to check for the normality of the data. Data of normal distribution and homogeneity of variance was tested with use of unpaired *t*-test or one-way ANOVA and Bonferroni post-hoc analysis and presented as mean ± standard deviation ($\bar{x} \pm s$). In cases, where dependent variables failed assumptions normality or equality of variance, data was analyzed by Kruskal-Wallis test followed by the Mann-Whitney *U* test and expressed as median (lower quartile–upper quartile, QL–QU). All *P* values shown are two-tailed. Statistical significance was set at a level of 0.05.

RESULTS

EH Model Established by Applications of AVP

Figure 1A shows typical light microscopic images of cochleae in group 1 (a) and group 2 (b). EH was not evident in ears of group 1. The Reissner's membrane was almost straight without extension, and the angle between Reissner's membrane and basilar membrane was approximately 45° . Conversely, displacement of the Reissner's membrane with bulge to scala vestibuli in ears of group 2 was noted.

R value were 0.30 ± 0.04, and 0.39 ± 0.03 in groups 1 and 2, respectively. R value in group 2 showed a significant increase compared with that in group 1 (*t*=–4.114, *P*=0.003, Figure 1B).

Effects of EA on EH

Figure 1A shows typical light microscopic images of cochleae in group 3 (c) and group 4 (d). Distinct hydrops of scala media was observed in group 3. Cochlear hydrops was found in group 4, but the distension of Reissner's membrane was less obvious in comparison to group 3. Meanwhile, foldings of Reissner's membrane were noted in group 4.

R value were 0.42 ± 0.02, and 0.36 ± 0.02 in groups 3 and 4, respectively. The value in group 3 was greater than that in group 2, but the difference was insignificant (*t*=2.224, *P*=0.057). By contrast, R value in group 4 was significantly less than that in group 3 (*t*=5.297, *P*=0.001, Figure 1B).

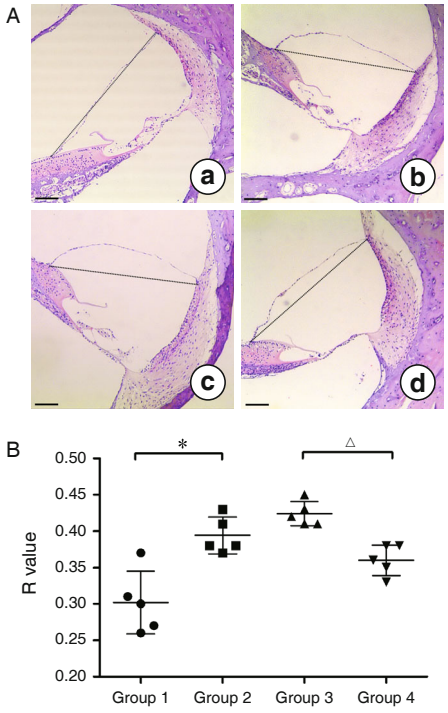

Figure 1. EA Reduces Cochlear Hydrops in EH Guinea Pigs ($\bar{x} \pm s$, *n*=5 in each group)

Notes: (A) Representative images of the midmodiolar section of guinea pig cochlea (×50). a: group 1; b: group 2; c: group 3; d: group 4. Bar=100 μm. The broken lines represent the normal position of the Reissner's membrane. (B) The ratio of SM area to SM + SV area (R value) of cochlea. $^{*}P$<0.01, compared with group 1; $^{\triangle}P$<0.01, compared with group 3.

Effects of EA on p-AVP Concentrations

Medians (QL–QU) levels of p-AVP were 0.16 (0.07–0.20), 0.24 (0.20–0.40), and 0.16 (0.14–0.20) in groups 5, 6, and 7, respectively. Guinea pigs in group 6, which serve as an important model control, showed significant increase from group 5 in p-AVP concentrations (Z=–2.626, *P*=0.009). In contrast, animals in group 7 showed a lower concentration of p-AVP than that in group 6, and this difference reached statistical significance (Z=–2.733, *P*=0.006, Figure 2A).

Effects of EA on Cochlear cAMP Levels

Cochlear cAMP levels were 6.04 ± 1.06, 7.93 ± 0.72, and 5.96 ± 1.22 in groups 5, 6, and 7,

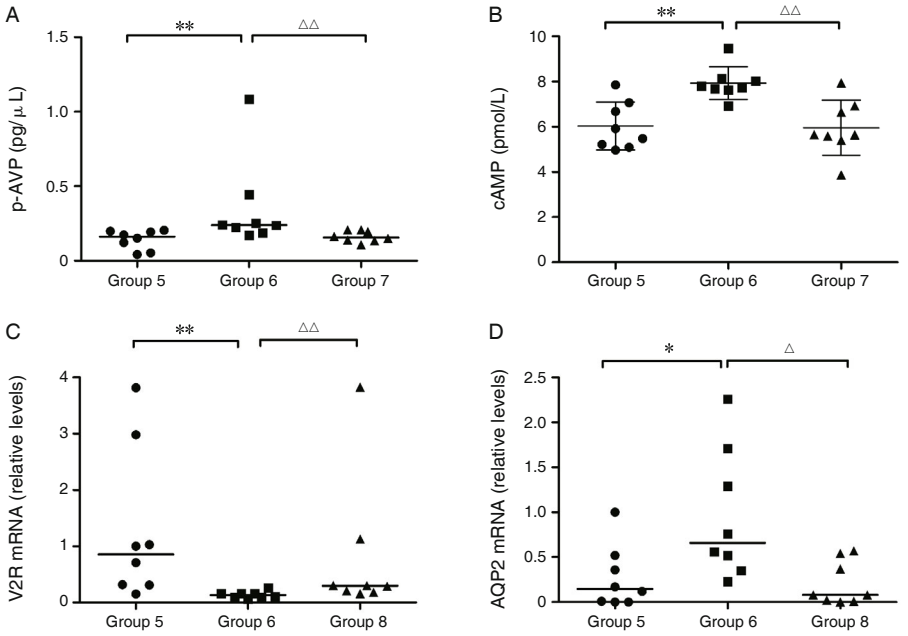

Figure 2. Effects of EA on p-AVP Levels, cAMP Concentration, V2R and AQP2 mRNA Levels in EH Guinea Pigs (*n*=8 in Each Group)

Notes: (A, C, D) Line at median; (B) Values are $\bar{x} \pm s$; $^{*}P<0.05$, $^{**}P<0.01$, compared with group 5; $^{\triangle}P<0.05$, $^{\triangle\triangle}P<0.01$ compared with group 6

respectively. One-way ANOVA demonstrated that the differences among three groups were statistically significant (F=9.546, P=0.001). The level of cAMP in group 6 showed a statistically highly significant increase in comparison to the group 5 (P=0.004). After EA treatment, the average concentration of cochlear cAMP in group 7 was lower than that of group 6 (P=0.003, Figure 2B).

Effects of EA on Cochlear V2R, AQP2 mRNA Expression

Medians (QL–QU) V2R mRNA levels were 0.86 (0.32–2.01), 0.14 (0.09–0.16), and 0.30 (0.20–0.72) in groups 5, 6, and 8, respectively. qRT-PCR results showed high levels of V2R mRNA expression in group 5. V2R mRNA expression was significantly decreased in guinea pigs of group 6 when compared with group 5 (Z=–2.951, P=0.003). In contrast, EA treatment significantly up-regulated the expression of V2R mRNA, and the difference reached statistical significance in comparison to group 6 (Z=–2.914, P=0.004, Figure 2C).

Medians (QL–QU) AQP2 mRNA levels were 0.15 (0.01–0.44), 0.66 (0.44–1.5), and 0.08 (0.02–0.46) in groups 5, 6, and 8, respectively. AQP2 mRNA was expressed in guinea pigs of group 5. AQP2 mRNA expression in group 6 was significantly higher than group 5 (Z=–2.366, P=0.018). After EA stimulation, AQP2 mRNA levels were significantly decreased as compared with group 6 (Z=–2.417, P=0.016, Figure 2D).

DISCUSSION

The present study confirmed the dehydration effects of EA against AVP-induced EH reflected by reduced ratio of SM area to SM + SV area (R value) in the cochlea. The value in group 4 was significantly less than that in groups 3. Moreover, we found that EA at GV 20 and SI 19 points significantly decreased AVP-induced elevated p-AVP levels and cochlear cAMP levels, attenuated the AQP2 mRNA expression, and up-regulated the expression of V2R mRNA in the cochlea. Thus, these observations indicated that the water homeostasis in the guinea pig inner ear might be partially regulated by EA via AVP-AQP2 pathway.

Accumulating evidence has been presented which supports the possibilities that AVP play a precise role in the formation of EH. Ultrastructural studies have shown an enlargement of the rat intrastrial space was caused by acute administration of AVP.[12] Therefore, it is likely that increased circulating levels of AVP increased production of endolymph, and eventually resulted in endolymph volume expansion. In addition, Takeda, et al[8] and Kitano[13] have shown that the chronic administration

of AVP via subcutaneously implanted osmotic-mini pumps resulted in EH in guinea pigs and rats. Based on AVP-induced model of hydrops by Takeda, et al[8] a recent study demonstrated that in guinea pigs given AVP intraperitoneally for 7 days showed an increase in endolymph volume.[9] Consistent with these studies, we have demonstrated that EH was detected after intraperitoneal injection of AVP for 1 week. Taking these results together, the question that arises here is how AVP contributes to EH.

It is well described that activation of the AVP-AQP2 pathway plays a role in edema-forming conditions such as liver cirrhosis, congestive heart failure (CHF), and pregnancy.[14] Recently, many clinical and experimental lines of evidence have demonstrated that the AVP-AQP2 pathway within the inner ear has been implicated in water reabsorption and plays a pivotal role in the formation of EH in Meniere's disease.[2] Several clinical trials have shown an increased circulating level of AVP in Meniere's disease patients.[15,16] In EH guinea pig model, dose-dependent studies showed an increased concentration of plasma AVP in response to [Arg]8 -VP administration, and elevated AVP levels have been suggested to be responsible for pathological fluid retention in the inner ear.[8] Consistently, the present study indicated an increased p-AVP levels in guinea pigs with EH. This increased p-AVP level in EH model may either be explained by the continuous AVP administration or nonosmotic stimuli to AVP release resembling nonosmotic-stimulated AVP secretion that occurs in other edematous conditions such as cirrhosis and pregnancy.[14] Consequently, the local decreases of V2 receptor mRNA levels, increases of cAMP accumulation and AQP2 mRNA levels may be associated with AVP stimulation *in vivo*.[3] Data obtained in rats showed an expression of V2 receptor mRNA in the cochlea,[17] and a down-regulation of that after chronic application of AVP.[18] In another study, Kitahara, et al[16] showed that cAMP activity of endolymphatic sac was basally up-regulated and cAMP sensitivity to AVP *in vitro* was largely increased in Meniere's patients. AQP2, AVP-regulated water channel protein, is strongly involved in conditions associated with impaired water excretion.[19] Molecular biological study has shown that the AQP2 mRNA levels in the cochlea were up-regulated by AVP stimulation.[20] Consistent with these previous studies, we have shown that over activation of AVP-AQP2 pathway in hydropic cochlea was detected after AVP injection.

In our study, we examined the aquaretic effect of EA, which has proven effective and safe in alleviation of edema.[21] Data indicated that EA has significantly reduced cochlear hydrops induced by AVP administration. This is supported by our previous studies reporting that EA significantly suppresses the development of EH compared to model control in aldosterone-injected guinea pigs.[5,6] A previous study reported that acupuncture can prevent activation of the hypothalamic-pituitary-adrenal (HPA) system and regulate the AVP immunoreactivity in the hypothalamic paraventricular nucleus of maternal separation rat pups.[22] Similar with this, EA treatment reduced the increase of p-AVP in our EH guinea pigs. Meanwhile, an elevated V2R mRNA level, decreased cAMP levels and AQP2 mRNA expression in our study might be associated with down-regulated AVP secretion by EA. These results indicated that repeated EA significantly suppressed the AVP-induced activation of AVP-AQP2 pathway, thereby down-regulate the expression of AQP2 mRNA in guinea pigs. Therefore, a decrease in AQP2 expression may contribute to a reduction in the endolymphatic volume of hydropic cochlea.

In conclusion, EA has dehydration effects on AVP-induced EH, which might be, at least in part, associated with its regulation of AVP-AQP2 pathway. AVP-AQP2 pathway represents a promising target for the treatment of Meniere's disease by EA.

Conflict of Interest

The authors declared no conflict of interests.

Author Contributions

The conception and study design: Chen HD, Jiang LY, He JJ, Chen XX; acquisition, analysis and interpretation of data: Jiang LY, Sun XJ, Wang XZ, Zhong S; drafting and revising the article critically: Jiang LY, Chen HD, He JJ.

REFERENCES

1. Hallpike CS, Cairns H. Observations on the pathology of Meniere's syndrome. Proc Roy Soc Med 1938;31:1317-1331.
2. Takeda T, Takeda S, Kakigi A, Okada T, Nishioka R, Taguchi D, et al. Hormonal aspects of Meniere's disease on the basis of clinical and experimental studies. ORL J Otorhinolaryngol Relat Spec 2010;71:1-9.
3. Beitz E, Zenner HP, Schultz JE. Aquaporin-mediated

fluid regulation in the inner ear. Cell Mol Neurobiol 2003;23:315-329.
4. Zhang J, Song JG, Xu J, Chen XM, Yin PH, Lv X, et al. ERK1/2-Egr-1 Signaling pathway-mediated protective effects of electroacupuncture in a mouse model of myocardial ischemia-reperfusion. Evid Based Complem Alternat Med 2014;2014:1-11.
5. Jiang LY, Ni FY, Wang CJ, Chen HD. Effects of acupuncture, moxibustion and electro-acupuncture on the hearing and morphology of cochlea in guinea pig models of endolymphatic hydrops. Chin J Compar Med (Chin) 2015;25:22-25.
6. Jiang LY, Wang CJ, Ni FY, Chen HD. Effects of electroacupuncture on cochlea morphology and expression of aquaporins in guinea pigs with endolymphatic hydrops. Chin Acupunct Moxibust (Chin) 2015;35:579-584.
7. Long AF, Xing M, Morgan K, Brettle A. Exploring the evidence base for acupuncture in the treatment of Meniere's syndrome—A systematic review. Evid Based Complem Alternat Med 2011;2011:1-13.
8. Takeda T, Takeda S, Kitano H, Okada T, Kakigi A. Endolymphatic hydrops induced by chronic administration of vasopressin. Hearing Res 2000;140:1-6.
9. Ma HZ, Zhong SX, Wen Y. The regulatory functions of aquaporin 2 by deamino arginine vasopressin in cochleas of guinea pigs. J Audiol Speech Pathol (Chin) 2014;22:286-289.
10. Naganuma H, Kawahara K, Tokumasu K, Satoh R, Okamoto M. Effects of arginine vasopressin on auditory brainstem response and cochlear morphology in rats. Auris Nasus Larynx 2014;41:249-254.
11. Klis SFL, Buijs J, Smoorenburg GF. Quantification of the relation between electrophysiologic and morphologic changes in experimental endolymphatic hydrops. Ann Otol Rhinol Laryngol 1990;99:566-570.
12. Nishimura M, Kakigi A, Takeda T, Okada T, Doi K. Time course changes of vasopressin-induced enlargement of the rat intrastrial space and the effects of a vasopressin type 2 antagonist. Acta Oto-Laryngologica 2009;129:709-715.
13. Kitano H, Takeda T, Takeda S, Suzuki M, Kitanishi T, Kitajima K, et al. Endolymphatic hydrops by administration of vasopressin in the rat. Acta Histochem Cytochem 2001;34:229-233.
14. Schrier RW, Cadnapaphornchai MA, Ohara M. Water retention and aquaporins in heart failure, liver disease and pregnancy. J Royal Society Med 2001;94:265-269.
15. Sawada S, Takeda T, Saito H. Antidiuretic hormone and psychosomatic aspects in Meniere's disease. Acta Otolaryngol Suppl 1997;528:109-112.
16. Kitahara T, Doi K, Maekawa C, Kizawa K, Horii A, Kubo T et al. Meniere's attacks occur in the inner ear with excessive vasopressin type-2 receptors. J Neuroendocrinol 2008;20:1295-1300.
17. Furuta H, Luo L, Ryan AF, Mori N. Expression of mRNA encoding vasopressin V1a, vasopressin V2, and ANP-B receptors in the rat cochlea. Hear Res 1998;117:140-148.
18. Kitano H, Suzuki M, Kitanishi T, Yazawa Y, Kitajima K, Isono T, et al. Regulation of inner ear fluid in the rat by vasopressin. Neuroreport 1999;10:1205-1207.
19. Boone M, Deen PMT. Physiology and pathophysiology of the vasopressin-regulated renal water reabsorption. Pflugers Arch Eur J Physiol 2008;456:1005-1024.
20. Sawada S, Takeda T, Kitano H, Takeuchi S, Kakigi A, Azuma H. Aquaporin-2 regulation by vasopressin in the rat inner ear. Neuroreport 2002;13:1127-1129.
21. Tian WQ, Peng YG, Cui SY, Yao FZ, Li BG. Effects of electroacupuncture of different intensities on energy metabolism of mitochondria of brain cells in rats with cerebral ischemia-reperfusion injury. Chin J Integr Med 2015;21:618-623.
22. Park HJ, Park HJ, Chae Y, Kim JW, Lee H, Chung JH. Effect of acupuncture on hypothalamic-pituitary-adrenal system in maternal separation rats. Cell Mol Neurobiol 2011;31:1123-1127.

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