Aquaporin-2 regulation by vasopressin in the rat inner ear

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Our previous studies have suggested a close relationship between vasopressin and endolymphatic hydrops, or the increased volume of endolymph in the inner ear. Endolymphatic hydrops is also thought to occur in Ménière’s disease patients. In the kidney collecting duct, vasopressin induces the expression of aquaporin-2 (AQP2), resulting in increased water reabsorption. We explored the possibility, using a quantitative PCR method, that vasopressin regulates the expression of AQP2 mRNA in the rat inner ear, as it does in the kidney. The levels of AQP2 mRNA in the cochlea and endolymphatic sac were significantly higher in rats treated with vasopressin than the levels in control animals. We speculate that over-expression of AQP2 may be involved in the formation of endolymphatic hydrops.

Key words: Aquaporin-2; Cochlea; Endolymphatic hydrops; Endolymphatic sac; Ménière’s disease; Vasopressin

INTRODUCTION

Aquaporins (AQPs) facilitate osmotic water transport across plasma membranes. AQP2, one of several isoforms of AQPs, is expressed in epithelial cell apical membranes in the collecting duct of the kidney [1,2]. AQP2 expression in the collecting duct is regulated by vasopressin via the type-2 vasopressin receptor. The function of AQP2 in the kidney is to regulate water reabsorption and plasma osmolarity. In the inner ear, AQP2 expression has been reported in the endolymphatic sac of rats and cochlea of immature mice [3,4]. However, there have been no reports that AQP2 is expressed in the cochlea of adult rats and its functions in the inner ear have not yet been determined.

It is generally accepted that the endolymph in the cochlea is produced by the stria vascularis, and that the endolymphatic sac is involved in regulatory mechanisms, such as absorption of endolymph [5,6]. The endolymph is unique because its potassium concentration is as high as that in intracellular fluids. Hair cell apical membranes are exposed to endolymph, and when stimulated, potassium currents flow through hair cells from the endolymphatic side to the perilymphatic side. Potassium currents depolarize hair cells, causing them to release neurotransmitter. Therefore, endolymph is thought to be essential for the normal function of hair cells. An increased volume of endolymph (endolymphatic hydrops) has been found in temporal bone specimens of patients with Ménière’s disease [7]. Endolymphatic hydrops is caused by endolymph production and absorption disequilibrium. However, the mechanisms underlying the occurrence of this disequilibrium have not yet been clarified.

It has recently been proposed that hormones such as vasopressin [8,9], aldosterone [10,11] and natriuretic peptide [12–14] may be involved in homeostatic mechanisms in the inner ear. We have reported that the vasopressin concentration in the blood of Ménière’s disease patients is elevated [15], that vasopressin administration causes endolymphatic hydrops in guinea pigs, and that type-2 vasopressin receptor mRNA is expressed in the rat inner ear [8,9]. These findings suggest that vasopressin may play a critical role in endolymph homeostasis. We speculate that vasopressin might regulate endolymphatic volume via AQP2 as it does in the kidney. The purpose of this study was to determine, using a quantitative PCR method, whether the levels of AQP2 mRNA in the rat cochlea and endolymphatic sac are regulated by vasopressin.

MATERIALS AND METHODS

Animals and preparation of specimens: Sixteen healthy male Wistar rats (200–300 g) were used in this experiment. Eight animals served as controls while the others received an intraperitoneal injection of 50 µg/kg arginine-vasopressin (AVP; V9879, Sigma, St. Louis, MO, USA). Three hours after AVP injection, the animals were deeply anesthetized with pentobarbital sodium (100 mg/kg, i.p.) and perfused with phosphate-buffered saline via the left ventricle to wash out the blood. Control rats were also anesthetized and perfused with phosphate-buffered saline. The cochlea, endolymphatic sac and kidney were dissected under a stereomicroscope. This study was approved by the Kochi Medical School Animal Care and Use Committee, which
RT-PCR: mRNA was purified using the QuickPrep Micro mRNA purification kit (Pharmacia, Piscataway, NJ, USA). Reverse transcription was performed using the SuperScriptII kit (Promega, Madison, WI, USA). RT-PCR was performed using HotStar Taq (Qiagen, Hilden, Germany). The primers for detection of AQP2 cDNA were 5'-TGGGTGGAATGGCCAGCTCTT3' (sense, corresponding to nucleotides 168–190) and 5'-ATGGGACAGGGCACGGCTACCC3' (antisense, corresponding to nucleotides 595–614; GenBank, accession no. D13906). The expected length of the PCR product was 447 bp. PCR, carried out using a thermal cycler (TP3000, Takara, Otsu, Shiga, Japan). The procedure was performed as follows: an initial denaturing period of 10 min at 94 °C, followed by 35 cycles at 94 °C for 1 min; 55 °C for 1 min and a final extension period of 10 min at 72 °C. PCR products were electrophoresed on a 3% agarose gel and stained with ethidium bromide. The PCR products were sequenced directly using ABI PRISM 310 (PE Applied Biosystems, Foster City, CA, USA) with the BigDye terminator cycle sequencing kit (PE Applied Biosystems).

Real-time PCR: Real-time PCR with the Roche LightCycler system (Roche Diagnostics, Biochemicals, Mannheim, Germany) was performed in a reaction mixture of 20 μl. This mixture contained 11.6 μl H2O, 2.4 μl MgCl2, 0.5 μmol of each primer solution, 2 μl cDNA solution and 2 μl LightCycler DNA Master SYBR Green I solution (Roche Diagnostics). The final concentration of MgCl2 in the reaction mixture was 4 mM. The primer pairs for AQP2 were the same as mentioned above; the pairs for β-actin were 5'-TGATTCTGAGGCACCCCTTT3' (sense, nucleotides 131–155) and 5'-ATGGGACAGGGCACGGCTACCC3' (antisense, nucleotides 395–372; GenBank, accession no. NM 012909). The PCR product was not obtained when RT was omitted (negative controls), indicating that the PCR product was derived from mRNA, not from genomic DNA.

To examine the possibility that the expression of AQP2 mRNA in the inner ear is regulated by vasopressin, we compared the amount of mRNA from AVP treated and untreated rats. The AVP treatment resulted in increases in M AQP2/M β-actin from 1.00 ± 0.20 to 1.98 ± 0.56 in the cochlea, from 1.00 ± 0.13 to 2.20 ± 0.40 in the endolymphatic sac, and from 1.00 ± 0.17 to 2.29 ± 0.32 in the kidney (Fig. 2). All the above increases were statistically significant (t-test, p < 0.01).

RESULTS

RT-PCR analyses revealed that AQP2 mRNA was expressed in the rat cochlea, endolymphatic sac, and kidney (Fig. 1). PCR products obtained from these tissues were of the expected size of 447 bp. The nucleotide sequence of the band agreed completely with the known sequence of rat AQP2 (GenBank no. NM 012909). The PCR product was not obtained when RT was omitted (negative controls), indicating that the PCR product was derived from mRNA, not from genomic DNA.

DISCUSSION

Our results indicate that the expression of AQP2 mRNA in the cochlea and the endolymphatic sac is up-regulated by AVP, just as it is in the kidney [16]. Several lines of evidence were verified. The AQP2 mRNA level (M AQP2) and β-actin mRNA level (M β-actin) were determined as the cDNA concentration from each CP in the standard curves of AQP2 and β-actin.

Data analysis: The expression level of AQP2 mRNA was normalized to the ratio of AQP2 and β-actin mRNA (M AQP2/M β-actin). To evaluate the effect of AVP administration on the expression of AQP2 mRNA, M AQP2/M β-actin values from tissue specimens (cochlea, endolymphatic sac, and kidney) from AVP-injected animals were normalized to the mean M AQP2/M β-actin values from control specimens. Statistical analysis was performed using the unpaired t-test following confirmation of equal variances by Levene’s test. All data are expressed as means ± s.e.m.
suggest a close relationship between vasopressin and endolymphatic hydrops: (i) the type-2 vasopressin receptor is expressed in the rat inner ear [8,9]; (ii) chronic vasopressin administration induces endolymphatic hydrops in the guinea pig cochlea [17]; and (iii) the vasopressin concentration in the blood of Ménière’s patients is elevated [15]. In view of the above reports and the vasopressin-induced up-regulation of AQP2 expression in the cochlea and the endolymphatic sac (Fig. 2), we speculate that over-expression of AQP2 may be involved in the formation of endolymphatic hydrops. However, the role of AQP2 in the cochlea and endolymphatic sac is still unclear. It has been reported that vasopressin suppresses vesicular transport in cultured epithelial cells dissected from the endolymphatic sac [3], but the relationship between vesicular transport and endolymphatic hydrops has not yet been clarified.

CONCLUSION
Aquaporin-2 (AQP2) mRNA is expressed in the rat cochlea and endolymphatic sac via RT-PCR. Vasopressin induces up-regulation of AQP2 mRNA in both the cochlea and endolymphatic sac, as it does in the kidney. These results support the suggestion that vasopressin may play a critical role in endolymph homeostasis and the formation of endolymphatic hydrops.

REFERENCES

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