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Short Communication

Effect of water deprivation on aquaporin 4 (AQP4) mRNA expression in chickens (*Gallus domesticus*)☆

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Abstract

Aquaporin (AQP) 4 is a member of the AQP gene family of water-selective transport proteins. We studied the effect of water deprivation on AQP4 gene expression in chickens. The nucleotide sequence of a chicken aquaporin 4 (AQP4) cDNA that encodes a protein of 335 amino acids showed high homology to mammalian AQP4. Using Northern blotting analysis, AQP4 mRNA in chickens was observed as a band of approximately 5.5 kb in several tissues in addition to the hypothalamus, proventriculus, kidney, and breast muscle. Quantitative analysis by real-time RT-PCR analysis showed that the mRNA expression of AQP4 in the hypothalamus significantly increased after dehydration. On the other hand, the mRNA expression of AQP4 in the kidney significantly decreased after dehydration. This suggests that AQP4 may play a pivotal role in osmoregulation in the chicken brain.

Theme: Endocrine and autonomic regulation
Topic: Osmotic and thermal regulation

Keywords: Chicken; Aquaporin 4 (AQP4); cDNA cloning; Water deprivation; Real-time PCR; Gene expression

Aquaporins (AQP) are water-selective transport proteins that confer high-membrane water permeability to certain tissues in animals, plants, and microorganisms. AQP4 cDNA has been isolated in rats [8,11], humans [13], mice [14], and bovines [22]. Mammalian AQP4 is characterized by its high water permeability and mercurial-insensitive water channel [25]. The primary expression sites of AQP4 are glia limitans, the epdymallining system, cerebellum, hippocampal dentate gyrus, and supraoptic and periventricular nuclei in the hypothalamus [3,11]. Recent studies on brain AQP4 have revealed the important role of AQP4 in brain water homeostasis. AQP4 is involved in blood–brain barrier (BBB) development, function, and integrity [17]. AQP4 knockout mice studies showed that AQP4 plays a key role in osmotically driven water transport and the development of cytotoxic brain edema and participates in the absorption of excess brain water and the resolution of vasogenic brain edema [15]. AQP4 is also proposed as a mechanism of central osmoreception corresponding to Verney's hypothalamic 'vesicular osmometers' [2,11,19]. In several peripheral tissues and cells, there are several reports that the gene expression of AQP1, 2, 3, and 5 is increased by hyperosmotic stress [6,9,10,12,16,23,26]; however, there is an in vitro report that AQP4 and 9 mRNA levels are induced in the brain after hyperosmotic mannitol stress [1].

In birds, the quail AQP4 cDNA sequence was recently cited in the computer database (accession number X80232), but little is known about the gene expression of AQP4. The purpose of this study was therefore to examine the gene expression of AQP4 in relation to water deprivation in chickens. First, as the quail AQP4 cDNA sequence contains only an open-reading frame and does not include the untranslated 3′ region, we cloned a full-length nucleotide

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sequence of the chicken AQP4 cDNA homologue. Second, we examined the tissue distribution of AQP4 mRNA using Northern blotting and analyzed the changes in AQP4 mRNA levels after 2 days of water deprivation treatment by real-time RT-PCR.

One-day-old male White Leghorn chicks were obtained from a local commercial supplier (Chubu Kagaku Shizai Co., Ltd., Nagoya, Japan). The chicks were killed by decapitation, and brain tissues were collected, frozen in liquid nitrogen, and stored at −80 °C until RNA extraction. For the isolation of chicken AQP4 cDNA, total RNA was extracted from the hypothalamus using TRIzol™ reagent (Invitrogen, CA). The resulting pellet of total RNA was dissolved in water, and total RNA was measured by spectrophotometer at 260 nm. Reverse transcription was performed with 1 μg total RNA and poly-(T_{18}) using PowerScript (QIAGEN K.K., Tokyo, Japan).

PCR was performed using a pair of primers designed from the human AQP4 cDNA sequence, namely, the sense and antisense primers were 5′-CAC ATC AAC CCC GCT GTG ACG GT-3′ and 5′-CCA AAG GAT CGG GCG GGG TTC AT-3′, respectively. All subsequent PCR reaction steps were performed using ExTaq (Takara Bio. Inc., Kyoto) and a programmable thermocycler (GeneAmp2700, Applied Biosystems, Foster City, CA). The reaction was incubated for 30 cycles at 95 °C, 55 °C, and 72 °C each for 0.5 min, with an elongated step of 5 min during the first 95 °C and the final 72 °C. To obtain the 5′ and 3′ regions of chicken AQP4 cDNA, the RACE method was employed using a SMART RACE cDNA Amplification Kit (Clontech, CA). All PCR fragments were cloned into pGEM-T Easy vector (Promega, WI) for DNA sequencing.

One-day-old male white Leghorn chicks were obtained from a local commercial supplier (Chubu Kagaku Shizai, Co., Ltd., Nagoya, Japan). For Northern analysis, chicks were maintained with free access to food and water until 7 days old. They were killed by decapitation, and tissues (brain, lung, heart, liver, proventriculus, duodenum, rectum, kidney, and muscle) were collected. Total RNA was extracted from each tissue, and 25 μg of total RNA was fractionated in 1.0% formaldehyde-agarose gel electrophoresis with 50 V constant. After electrophoresis, agarose gel was stained with ethidium bromide. Total RNA was transferred to a nylon membrane (Hybond N^+, Amersham, UK) by capillary action.

The membranes were pre-hybridized with hybridization buffer (50% formamide, 5× SSC, 1× Denhardt's solution, 25 mg/ml salmon sperm DNA, and 10% dextran sulfate) for 2 h at 42 °C. After pre-hybridization, the membranes were incubated with hybridization buffer containing a [α^{32} P]-dCTP-labeled DNA probe for 18 h at 42 °C. The AVT cDNA insert, 270 bp of the distal 3′ glycopeptide part of the AVT gene, was labeled with [α^{32} P]-dCTP (ICN International) using a *Bca*BEST™ Labeling Kit (Takara Bio, Kyoto, Japan). After hybridization, these membranes were washed three times, with a final washing in 0.1× SSC containing 0.1% SDS at 60 °C for 15 min. The hybridized membranes were exposed to X-ray film with an intensifying screen (Amersham Inc., UK) for 2 to 7 days at −70 °C.

One-day-old male white leghorn chicks were maintained with free access to food and water until 7 days old, after which they were divided into two groups, (1) free access to food and water (control group) and (2) free access to food without water (dehydration group), and kept for 2 days. Tissue samples (hypothalamus, proventriculus, kidney, and breast muscle) were taken, and total RNA was extracted from the tissue. The extracted total RNA was treated with DNase and reverse transcribed into cDNA. Real-time PCR was carried out using a Smart Cycler II System (Takara Bio Inc., Japan) using Quantitect SYBR Green PCR (QIAGEN K.K., Japan). PCR reactions were subjected to the following thermal protocol: (97 °C × 5 s; 60 °C × 10 s; 72 °C × 30 s) × 50 cycles. The relative expression was analyzed according to the $\Delta\Delta C_t$ method using the S17 gene for normalization [27]. The chicken AQP4 primers were 5′-CCA TCG TGG GAG CTG GCA TCC TCT A-3′ and 5′-AAT GTA ATT ATC AGC TCC ACC AGG A-3′. The chicken arginine vasotocin (AVT) primers were 5′-GCA GTG AGC AGG CAG AAG AGG-3′ and 5′-GCT CAG AGG CCA GGC TGC TTG-3′ [7]. The chicken ribosome protein S17 primers were 5′-AAG CTG CAG GAG GAG GAG AGG-3′ and 5′-GGT TGG ACA GGC TGC CGA AGT-3′ [24].

All values are the mean ± SEM. Statistical differences were analyzed using the *t* test at $P < 0.05$.

Full-length cloned chicken AQP4 cDNA and deduced amino acid sequences are shown in Fig. 1. The cDNA was 1940 bp long and encoded a chicken AQP4 protein consisting of 335 amino acid residues. The predicted amino acid sequence of chicken AQP4 cDNA contains six transmembrane domains and two NPA motifs (Asn−Pro−Ala) preserved in the AQP4 cDNA of mammals [8,11,13,14,22]. The deduced amino acid sequence of chicken AQP4 shows 98.8% homology to that of quail AQP4 (accession number X80232) and 84.5% homology to human AQP4 [13] (Fig. 2). The avian-deduced amino acid sequence of AQP4 is 12 amino acids longer than that of mammals [8,11,13,14,22] (Fig. 2).

A single band of approximately 5.5 kb was detected in the chicken hypothalamus, proventriculus, kidney, and breast muscle (Fig. 3). There were no bands in the lung, heart, liver, duodenum, and rectum.

Two days of dehydration treatment significantly increased plasma osmolality in chickens (control group, 321.5 ± 2.4 mOsm; water deprivation group, 357.4 ± 3.4 mOsm, $P < 0.05$). Real-time RT-PCR analysis showed that mRNA levels of hypothalamus AVT increased after water deprivation treatment ($P < 0.05$), concurrently with a significant increase in AQP4 mRNA levels in the hypothalamus ($P < 0.05$) (Fig. 4). On the other hand, AQP4 mRNA levels in the kidney significantly decreased ($P < 0.05$). There were no significant differences in AQP4 mRNA levels in the proventriculus and breast muscle.