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Original investigation

Are There Any Sex-Depended Differences in Water Transporting Proteins of Choroid Plexus in Mice?

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ABSTRACT

Background: It is known that sex hormones show modulatory effects on the central nervous system (CNS). Various CNS pathologies show sex-related differences in terms of rates, symptoms, and the course of the disease. Some of the functions of choroid plexus (CP) are under the control of sex hormones. The aim of this descriptive study was to evaluate sex effects on CP water channels.

Methods: Expressions of Aquaporin (AQP) 1, 2 and 4 proteins in CP were compared between in male and female Balb/C mice brains; and also in different stages of the estrous cycle, with immunohistochemistry.

Results: No difference is observed for AQP 1, 2 and 4 immunoreactivities in male and female brain, including different stages of the estrous cycle.

Conclusion: In conclusion, to the best of our knowledge, AQP2 expression was shown in the CP for the first time, and no sex hormone-dependent differences were found in terms of expressions of AQP1, 2 and 4.

Keywords: choroid plexus, water channels, sex differences, aquaporin 1, aquaporin 2, aquaporin 4

INTRODUCTION

The choroid plexus (CP) is a special structure of the third, fourth, and lateral ventricles of the brain, which formed by the epithelial cell layer and basement membrane. Below the basement membrane, convoluted blood vessels and various cells lay in the connective tissue. CP secretes the cerebrospinal fluid (CSF) which is a liquid with a unique content around brain tissue. Epithelial cells develop from modified neuroepithelium of the CP's are connected by tight junctions which is a structure that controls bi-directional transport between the brain microenvironment and blood (1). In addition to the morphological barrier, there are also chemical barriers, exist to epithelial cells halting the paracellular movement of molecules into the central nervous system (CNS) (2).

CP, unlike most other formations of the CNS, is a structure that found in all vertebrates, from the lowest species to the human. CP also shows the similarity between species in terms of functions. The CP is very important for the formation, growth, and maintenance of the CNS (3).

It is known that sex hormones show modulatory effects on the CNS. Various CNS pathologies show sex-related differences in terms of rates, symptoms, and the course of the disease (3–5). As CP contains estrogen, progesterone, and androgen receptors, various functions that occur in the CP are under the control of sex hormones. However, there is very little information about the sex-dependent differences at the molecular level and the significance of these differences in CP (6).

Aquaporins (AQPs) are water-specific channels that are receiving increasing attention day by day. Thirteen AQP types (AQP 0–12) were defined in mammals until now (7). AQPs were divided into subgroups as classical (orthodox), aquaglyceroporins and unorthodox. This classification shows AQPs permeation specificities: orthodox or classical AQPs (AQP 0, 1, 2, 4, 5, 6, 8) are especially permeable to water; aquaglyceroporins (AQP3, 7, 9, 10) are permeable to both water and small solutes; and unorthodox AQPs (AQP 11, 12) that selectivities were not clearly shown (8). In the CNS, 7 of this AQP are shown *in vivo* or *in vitro* (9). AQP1 and 4 are found extensively in the brain and are thought to be

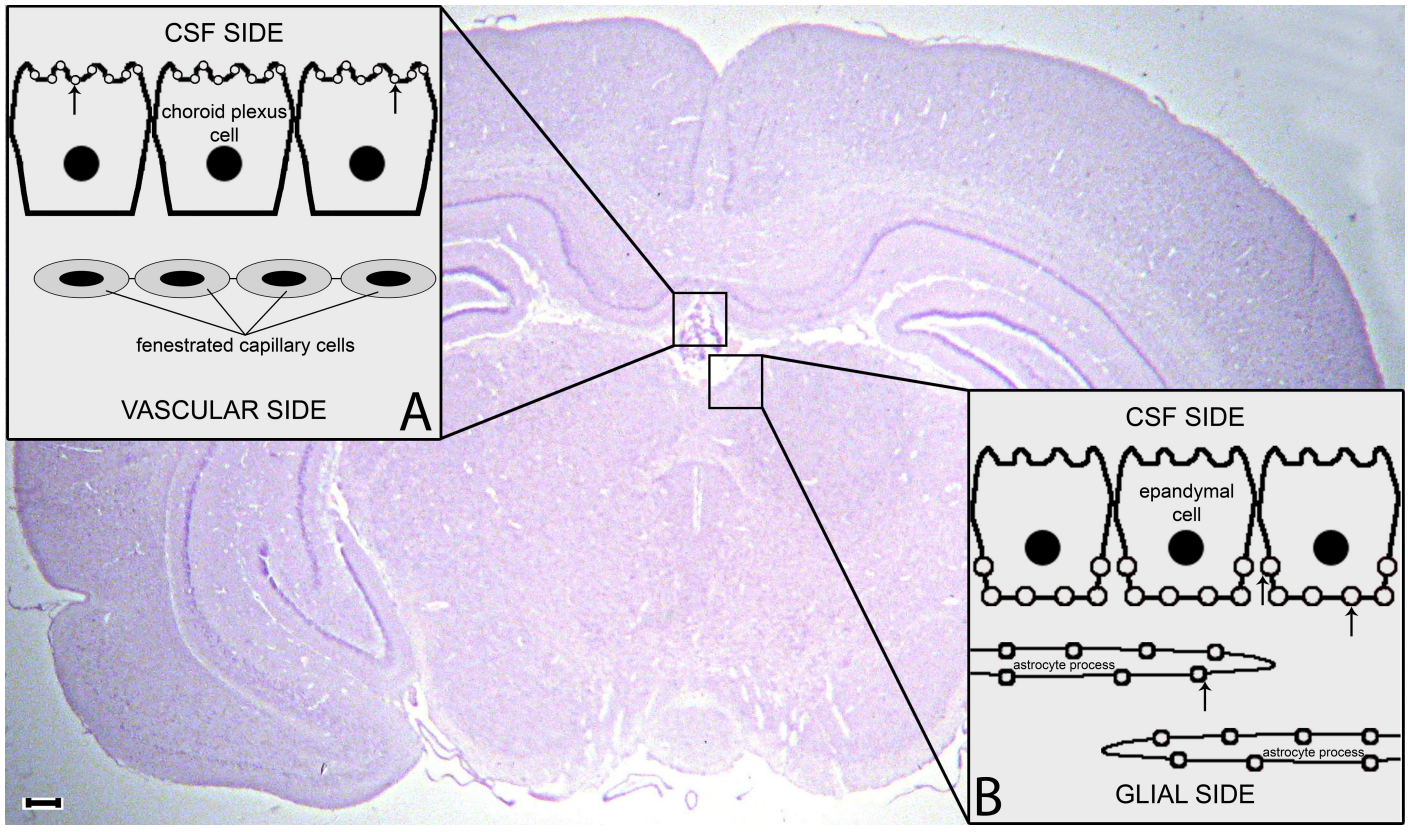

Figure 1 A, B. A representative picture is demonstrating a coronal section of the adult mouse brain. Distribution of AQP1 (arrows, a). Distribution of AQP4 (arrows, b) (Scale bar, 100 µm).

related to the regulation of water physiology in general, but the molecular pathways involved in their function have not been fully demonstrated. AQP1, the main water channel, is located within the CSF side of the CP epithelium (9–10) (Figure 1a). AQP2 is mainly found in the kidney tissue, but it also found in the ependymal cell layer. AQP2 has not been studied much in the CNS and, to our knowledge, it has not been shown in the CP to this day (11). AQP4 is located within glial membranes, mostly in subpial endfoot of the astrocytes, glial and lateral walls of the ependymal cell membranes (Figure 1b). Previously, it was reported that there was no sex-dependent difference in ovariectomized rats in CP in terms of AQP1 and 4 expressions. However, AQP5 and 9 expressions were reported to increase in ovariectomized rats (1).

Thus far, CP has been studied less than other structures in the central nervous system. However, the number of studies on CP is gradually increasing. AQP receptors have been the subject of various researches both in terms of location and function in recent years. Therefore, the aim of this descriptive study was to evaluate sex effects on CP water channels.

MATERIAL AND METHODS

Animals and tissue collection

The study included 25 mature females (8 weeks, n=20) and male (12 weeks, n=5) Balb/C mice. The animals were fed a standard laboratory diet and water and were housed in standard cages in an air-conditioned room with a 12-hour light-dark cycle, and constant temperature (20–22°C) and relative humidity (65–70%). All experimental protocols were approved by the Local Ethics Committee for Animal Research (Approval no/date: 05/2008). The study was carried out in the "Experimental and Research Laboratories."

The estrous cycle of mice divided into four categories: proestrus, estrus, metestrus, and diestrus (12). Five mice were included in each estrous cycle phase group. The phase of the estrous cycle of female mice was determined by vaginal smear. Vaginal secretions of the mice were obtained with a tip of plastic pipet and placed on a slide with a small amounth of phosphate buffered saline (PBS). Then, photomicrographs of vaginal secretions examined and phase of estrous cycle determined according to types of the cells (12).

For the brain collection, intracardiac perfusion with cold Somogyi's fixative was performed. Before the fixation, the intravascular blood was washed out with sterile% 0.09 NaCl solution (20 ml/min for 5 min). After the perfusion, the brains were extracted from the cranium and kept in the same fixative at 74 +4°C for 12 hours, dehydrated an increasing ethanol series, cleared with xylene and finally embedded in paraffin blocks. 5-µm thick coronal sections including the third ventricle (13) were collected onto poly-L-lysine-coated slides (Sigma-Aldrich, St. Louis, MO, USA) for immunohistochemistry staining.

Immunohistochemistry

For AQP1, AQP2 and AQP4 immunohistochemistry, sections were deparaffinized and washed in PBS twice and boiled in citrate buffer (10 mM; pH 6.0) for 7 minutes for antigen retrieval and blocked for endogenous peroxidase activity with methanol containing 3% H_2O_2 for 15 min. Slides were then incubated with universal blocking reagent (BioGenex, San Ramon, CA, USA) for nonspecific binding for seven minutes (room temperature). Next excess blocking solution was removed, and anti-rabbit AQP1 (sc-20810), AQP2 (sc-28629), AQP4 (sc-20812) (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) diluted in dilution buffer (1/200) were applied for overnight at +4°C in a humidified chamber. After several washing steps in PBS, sections were incubated with biotinylated goat anti-rabbit IgG (1/350 dilution Vector Lab, Burlingame, CA, USA) incubation for 1 hour at room temperature followed by LSAB streptavidin-peroxidase complex (Dako, Carpinteria, CA, USA) incubation for 20 min. and were rinsed with PBS. Antibody-antigen complexes were visualized by incubation with diaminobenzidine (DAB) chromogen (DAB, Vector Laboratories). Sections were counterstained with Mayer's hematoxylin (Sigma), dehydrated, mounted and examined by a Zeiss-Axioplan (Oberkochen, Germany) microscope.

Statistical Analysis

Micrographs were taken using SPOT Advanced 4.6 at 20X magnification. All these micrographs were analyzed with Image-J (Image Processing and Analysis in Java; US National Institutes of Health, Bethesda, MD; https://imagej.nih.gov/ij/) software by scanning 10 non-overlapping fields in each tissue. Statistical significance was determined by analysis of variance with the Dunnett posttest by Sigma Stat 3.5 (Systat Software, San Jose, CA) software. Data were considered statistically significant at $p<0.05$.

RESULTS

Expressions of AQP1, 2 and 4 proteins in CP were compared between in male and female brains; and also in different stages of the estrous cycle (Figure 2). The same AQP expression patterns in male and female CP's suggested that there is not any sex-dependent expression of water channels. No statistically significant difference ($p>0.05$) in the AQP expressions between the different phases of the estrous cycle indicates that AQP receptors in CP, are not under hormonal control (data not shown)

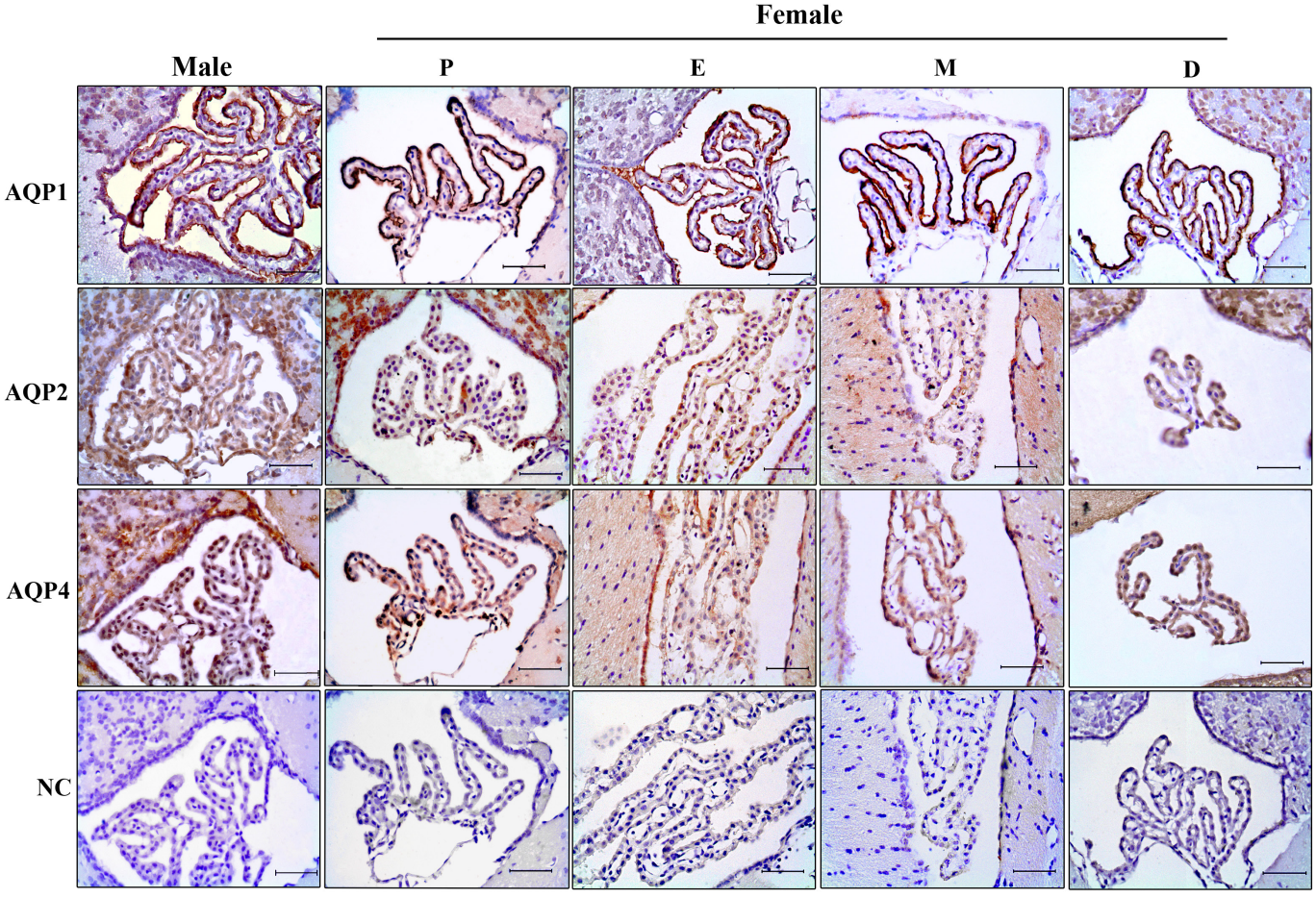

Figure 2. Immunohistochemical staining of AQP1, 2 and 4 in the male and female plexus choroideus. There was no significant difference for AQP1, 2 and 4 immunoreactivities in terms of sex and estrous cycle phases (P, proestrus; E, estrus; M, metestrus; D, diestrus; NC, negative control; Scale bar, 50 µm).

DISCUSSION

CP has a much more vascular texture than the other structures in the brain. CP can be thought of as the brain's kidney because it is responsible for CSF clearance (4). Among AQP subspecies, AQP 1 and 4 are in the classical aquaporin family and are primarily responsible for water transport (8, 10, 14).

AQP1 is abounded in the CP and is responsible for the regulation of CSF circulation (10). AQP4 is the most frequently studied AQP in the CNS and is responsible for water transfer, cell migration and stimulation of neurons. AQP4 is located within glial membranes, mostly in subpial endfoot of the astrocytes, glial and lateral walls of the ependymal cell membranes (15). The involvement of AQP4 in this critical localization between CNS-CSF suggests that it is involved in the transport of water through and out of the brain (16). AQP2 is mainly found in the kidney tissue, but it also found in the CNS, gastrointestinal system, urinary and genital systems. AQP2 has not been studied much in the CNS and, to our knowledge; it has not been shown in the CP to this day (11, 17). Since AQP1 and 4 are frequently shown on CP and then AQP2 is very important in water transport and has not been shown in the CP, we chose to study on these water channels.

Expressions of various AQPs in different tissues have been shown to controlled by hormones (1, 18–20). Herak-Kramberger et al. (21) found that post-pubertal AQP1 expressions in males were higher than females, in their study of sex differences in AQP1 in rat kidneys. They claim that this difference is due to high androgen, low progesterone exposure. Cheema et al. (22) have shown that AQP2 expressions were elevated in ovariectomized rat kidneys. They also have shown that estrogen replacement had decreased AQP2 levels. It is known that AQP1 and 2 are also located in the uterine wall, and their expressions are under hormonal control. AQP2 has been shown to increase with estrogen and progesterone effects in the mid-secretory phase of the menstrual cycle (23).

In a small number of studies, the various AQPs in the CP have been compared in terms of sex differences (1, 17). Since both CP and AQP are known to be under hormonal control, we compared expressions of AQP1, 2 and 4 proteins in CP, between the male and female brain and also expressions in different stages of the estrous cycle. CP showed no expressional difference in terms of AQP1, 2 and 4 proteins during the female estrous cycle and male brain as well. We detected AQP1 and 4 protein expressions as expected locations in choroid plexus cells without any fluctuation according to female estrous phases. These results suggest ovarian hormones have no effect on the expression of studied water channel proteins in CP cells.

Similarly, with our study, it was reported that there was no sex-dependent difference in ovariectomized rats in CP in terms of AQP1 and 4 expressions. However, AQP5 and 9 expressions were reported to increase in ovariectomized rats (1). Yaba et al. (24) studied AQP7 and 9 in CP in terms of expressions during the different estrous phases. Unlike our study, in this study, it was determined that AQP expressions differed during estrous phases. This difference may be due to the study of different AQPs.

In our study, AQPs were shown only by immunohistochemistry. This is the biggest limitation of our work. In the present literature, the AQP and CP relationships have been poorly studied, and the number of studies on sex has been negligible. For this reason, our work is novel.

In conclusion, to the best of our knowledge, in the CP, AQP2 was shown for the first time, and no sex-dependent difference was found in terms of AQP1, 2 and 4. Moreover, it was shown that AQP1, 2 and 4 expressions did not differ during the estrous cycle.

Informed Consent: Animal research

Compliance with Ethical Standards: The research was carried out after approval from the Akdeniz University Animal Care, Use and Animal Experiments Ethics Committee (No: 5/05, Date: 25.05. 2008).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - GT, ND, BS; Design - GT, ND, BS; Supervision - GT, ND, BS; Fundings - GT, ND, BS; Materials - BS, GT; Data Collection and/or Processing - BS, GA; Analysis and/or Interpretation - BS, GA; Literature Search - BS, GA; Writing Manuscript - BS, GA; Critical Review - GT, BS, GA

Conflict of Interest: No conflict of interest was declared by the authors.

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