RESEARCH ARTICLE

Molecular Reproduction & Development 81:524–530 (2014)

Oxytocin Regulates the Expression of Aquaporin 5 in the Late-Pregnant Rat Uterus

ESZTER DUCZA,* ADRIENN B. SERES, JUDIT HAJAGOS-TÓTH, GEORGE FALKAY, AND RÓBERT GÁSPÁR

Department of Pharmacodynamics and Biopharmacy, Faculty of Pharmacy, University of Szeged, Szeged, Hungary

SUMMARY

Aquaporins (AQPs) are integral membrane channels responsible for the transport of water across a cell membrane. Based on reports that AQPs are present and accumulate in the female reproductive tract late in pregnancy, our aim was to study the expression of AQP isoforms (AQP1, 2, 3, 5, 8, and 9) at the end of pregnancy in rat in order to determine if they play a role in parturition. Reverse-transcriptase PCR revealed that specific *Aqp* mRNAs were detectable in the myometrium of non-pregnant and late-pregnancy (Days 18, 20, 21, and 22 of pregnancy) rat uteri. The expression of *Aqp5* mRNA and protein were most pronounced on Days 18–21, and were dramatically decreased on Day 22 of pregnancy. In contrast, a significant increase was found in the level of *Aqp5* transcript in whole-blood samples on the last day of pregnancy. The effect of oxytocin on myometrial *Aqp5* expression in an organ bath was also investigated. The level of *Aqp5* mRNA significantly decreased 5 min after oxytocin (10^{-8} M) administration, similarly to its profile on the day of delivery; this effect was sensitive to the oxytocin antagonist atosiban. The vasopressin analog desmopressin (3.7×10^{-8} M), on the other hand, did not alter the expression of *Aqp5*, but did increased the amount of *Aqp2* mRNA, an effect that was atosiban-resistant. These results lead us to propose that oxytocin selectively influences the expression of *Aqp5* at the end of pregnancy, and may participate in events that lead to parturition in the rat. The sudden increase of AQP5 in the blood on the last day of pregnancy may serve as a marker that indicates the initiation of delivery.

*Corresponding author:
Department of Pharmacodynamics and Biopharmacy
University of Szeged, H-6720
Eötvös u. 6, Szeged 6270
Hungary.
E-mail: ducza@pharm.u-szeged.hu

Grant sponsor: János Bolyai Research Fellowship; Grant number: BO/00231/11

Mol. Reprod. Dev. 81: 524–530, 2014. © *2014 Wiley Periodicals, Inc.*

Received 7 January 2014; Accepted 7 March 2014

Published online 9 April 2014 in Wiley Online Library (wileyonlinelibrary.com).
DOI 10.1002/mrd.22320

INTRODUCTION

Water is the major component of cells and tissues, and water movement across the cell membrane is a fundamental property of life. It was long assumed that the transport of water was due to simple diffusion through the lipid bilayer that encloses cells; this picture changed when the first water channel, aquaporin (AQP)-1, was identified in human erythrocytes (Preston et al., 1992). The AQPs are a family of small (25–34 kDa), hydrophobic, integral-membrane, channel proteins that facilitate the rapid, passive movement of water across a membrane. To date, 13 isoforms of AQPs (AQP0–AQP12) have been identified in mammals. In view of the differences in coding sequence, permeability properties, and the findings of phylogenetic comparisons, AQP0–AQP10 are sub-divided into two major groups: classical AQPs and aquaglyceroporins (Agre et al., 2002; Agre and Kozono, 2003; Zardoya, 2005). Classical AQPs (AQP0, AQP1, AQP2, AQP4, AQP5, AQP6, and AQP8) are selective channels that are permeable to water, but not to small organic or inorganic molecules. Aquaglyceroporins

Abbreviations: AQP, aquaporin; OT, oxytocin.

(AQP3, AQP7, AQP9, and AQP10) are non-selective channels that allow the flow of glycerol, urea, and other small non-electrolytes as well as water. The functions of AQP11 and AQP12, referred to as super-aquaporins, are not clear. The physiological roles of AQPs have been deduced on the basis of experiments with *Aqp* knockout animals and human diseases, as specific inhibitors are not available (Skowronski, 2010).

Previous studies have shown that specific AQP isoforms are expressed in both the male and female reproductive tissues of the rat and mouse (Jablonski et al., 2003). The abundance of human endometrium AQP2 in the mid-secretory phase at the time of embryo implantation suggested that this isoform plays a role in uterine receptivity (He et al., 2006). It has also been postulated that AQPs participate in fertilization, blastocyst formation, and implantation (Richard et al., 2003). Multiple aquaporins are expressed in early-stage human embryos, while AQP3 and AQP7 play roles in pre-implantation mouse embryo development. Knockdown of either AQP3 or AQP7 levels by targeted siRNA injection into 2-cell mouse embryos significantly inhibited pre-implantation development (Xiong et al., 2013). Moreover, the expression and distribution of *Aqp3* is developmentally regulated from the 8-cell to blastocyst stage in mouse embryos before and after vitrification (Nong et al., 2013).

The cellular and sub-cellular localization of amniotic AQPs indicate that these channels also play distinct roles in water and urea transport, cell migration, cell proliferation, and apoptosis during amniotic fluid homeostasis and/or tissue remodeling of amniotic membranes (Kobayashi and Yasui, 2010). AQP1, 3, 8, 9, and 11, for example, play crucial roles in the transfer of water across the placenta. Mann et al. (2006) detected a significant increase in *AQP1* expression in the amnions of polyhydramnios individuals, whereas there were significant decreases in *AQP1* and *AQP3* in the amnion and chorion of oligohydramnios individuals. The expression of *AQP8* is significantly lower in the oligohydramnios placenta than in the normal-pregnancy placenta at term, and the expression of *AQP9* mRNA in the fetal membranes is higher in polyhydramnios individuals (Sha et al., 2011). *AQP11* expression has also been reported in the human amniotic membrane at term (Prat et al., 2012).

Data concerning the expression of AQPs in the late-pregnancy uterus and in relation to parturition have not been established. Consequently, the primary aim of this study was to examine the expression of AQPs in the late-pregnancy rat uterus. We additionally hoped to identify hormonal regulators of AQP expression during pregnancy, which we predict to be regulated by steroid sex hormones (Lindsay and Murphy, 2006, 2007). As oxytocin (OT) is one of the main hormones responsible for pregnant uterine contractions, we investigated whether or not OT has any effect on the expression of AQPs in late-pregnancy rat uteri. To assess specificity of action, we also evaluated how vasopressin, which regulates *AQP2* expression in the kidney (Wilson et al., 2013) and differs in sequence from OT by only two amino acids, affects uterine *Aqp* expression.

RESULTS

Reverse-transcriptase PCR investigations revealed that the mRNAs of the investigated *Aqp* isoforms (*Aqp1*, *2*, *3*, *5*, *8*, and *9*) were detectable in the myometrium of non-pregnant and late-pregnancy (Days 18, 20, 21, and 22 of pregnancy) rats. The peak of *Aqp1* mRNA expression, observed on Day 18, decreased towards the end of pregnancy (Day 22) (Fig. 1a). *Aqp2* and *Aqp8* transcripts increased significantly on Day 18 and remained high until Day 21, but declined to the level in the non-pregnant uteri by Day 22 (Fig. 1b and e). *Aqp5* mRNA was highest on pregnancy Days 18–21, followed by a dramatic decrease on the last day of pregnancy (Fig. 1d). The abundance of *Aqp3* and *Aqp9* mRNA remained lower than those in the non-pregnant uteri on all days investigated (Fig. 1c and f).

Given the timing of peak transcription, we further investigated the mRNA and protein expression of *Aqp5* in non-pregnant animals as well as periodically throughout pregnancy (Days 8, 12, 16, 18, 20, 21, and 22). Uterine

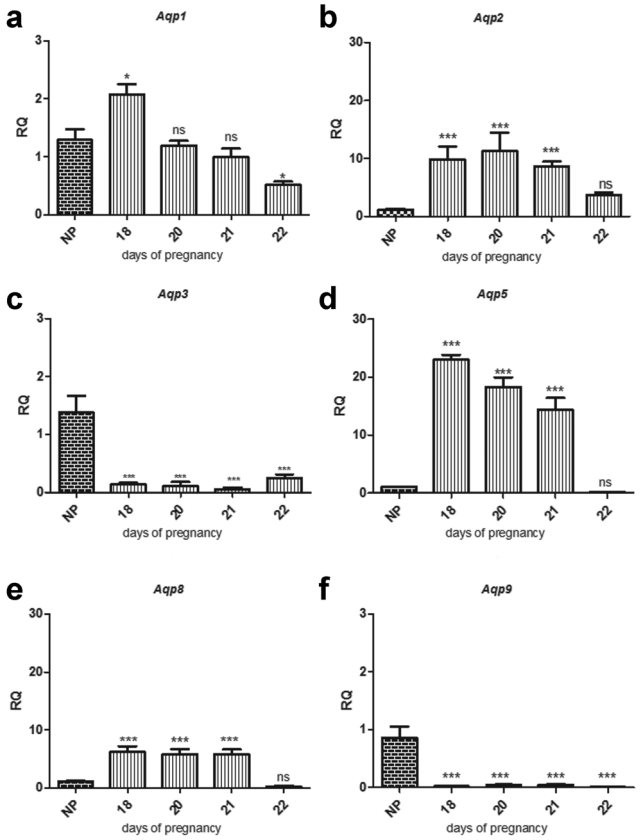

Figure 1. Reverse-transcriptase PCR analysis of *Aqp1* (**a**), *2* (**b**), *3* (**c**), *5* (**d**), *8* (**e**), and *9* (**f**) expression in late-pregnancy rat uteri (n = 6 per day). Changes in expression of *Aqp5* mRNA were most extensive, with a dramatic decrease on the day of birth. ns $P > 0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ as compared with the control, non-pregnant uterus (NP). RQ, relative quantity of mRNA compared to *Actb*. Each bar denotes the mean ± standard deviation.

Aqp5 mRNA abundance peaked on Day 18, while protein levels peaked on Day 20 (Fig. 2). In contrast, *Aqp5* mRNA and protein expression in the blood did not undergo significant changes until the final day of pregnancy (Fig 3).

In vitro pre-treatment of myometrium cross-sections with OT (striped column) caused an increase in uterine *Aqp2* expression on Day 18, but the OT antagonist atosiban (gridded column) prevented this effect (Fig. 4). *Aqp5* mRNA levels significantly decreased (striped columns) relative to the control uterus within 5 min after OT administration (Fig. 5), but this difference gradually declined such that no significant difference was found between the mRNA levels after 20 min. In the presence of atosiban, *Aqp5* mRNA abundance (dotted column) was still lower than the control, but it was significantly higher than the OT-treated samples (Fig. 5). Again, no differences were observed in the *Aqp5* mRNA levels as compared with the non-treated sample 10 or 20 min after atosiban treatment.

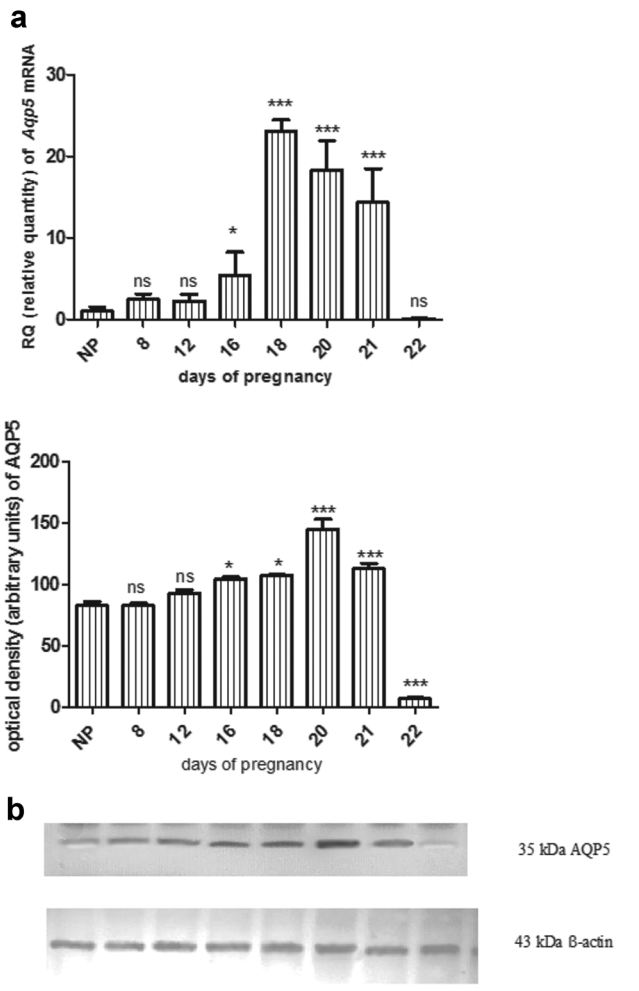

Figure 2. Reverse-transcriptase PCR and Western blot analysis of *Aqp5* on different days of pregnancy (n = 6 per day). Abundance of *Aqp5* mRNA (**a**) and AQP5 protein (**b**) was significantly decreased on the day of birth. ns $P > 0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ as compared with the control, non-pregnant uterus (NP). Each bar denotes the mean ± standard error.

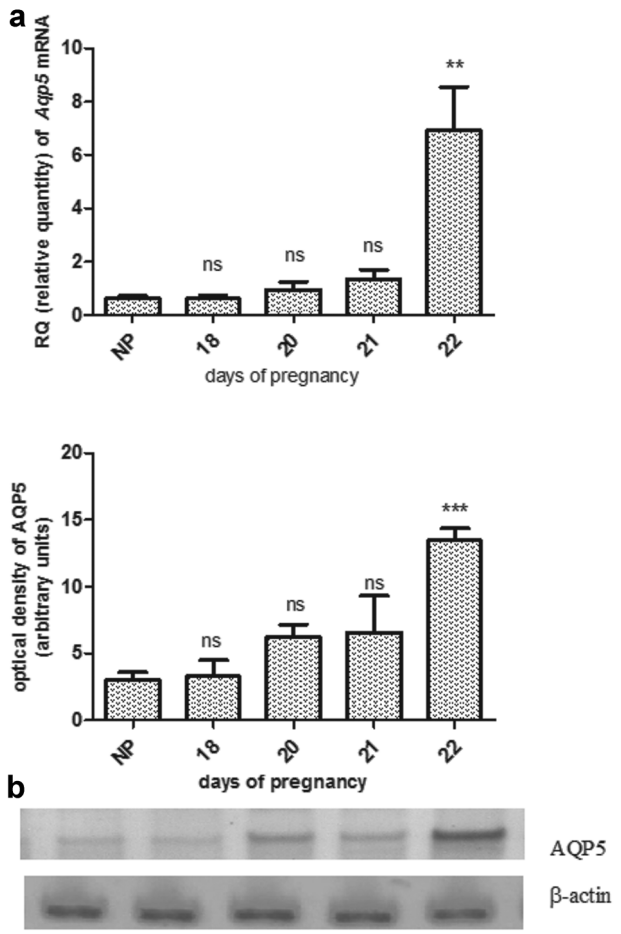

Figure 3. Reverse-transcriptase PCR (**a**) and Western blot analysis (**b**) of *Aqp5* expression in whole blood (n = 6 per day). Expression increased significantly on the day of birth. ns $P > 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ as compared with data on the previous day. Each bar denotes the mean ± standard deviation.

Desmopressin, an analog of vasopressin that differs from OT by only two amino acids, increased *Aqp2* mRNA levels on pregnancy Day 18 (Fig. 6a). This effect was antagonized by atosiban (Fig. 6a, gridded columns). In contrast, the level of *Aqp5* mRNA did not change following desmopressin treatment (Fig. 6b).

DISCUSSION

The AQPs are utilized by a wide variety of cells in organs such as the kidney, lung, pancreas, brain, gastrointestinal tract, eye, ear, muscles, uterus, and testis. For example, in the non-pregnant uterus, *Aqp5* expression increased in the apical plasma membrane of luminal epithelial cells in response to progesterone, providing a mechanism for the

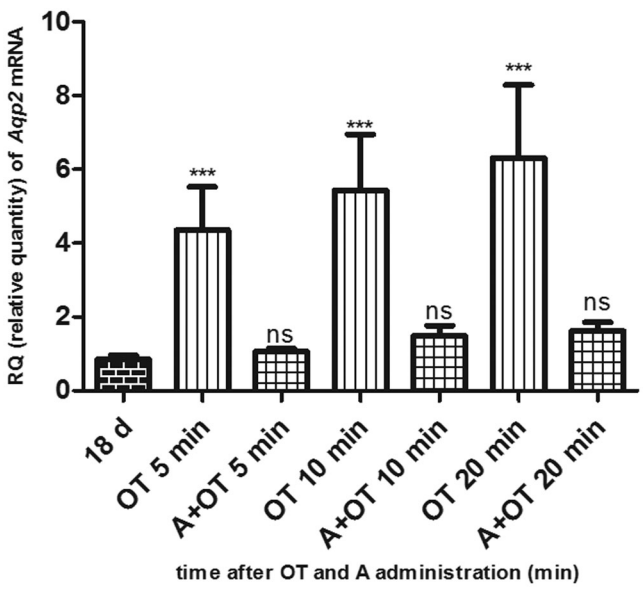

Figure 4. Changes in the expression of *Aqp2* mRNA on Day 18 of pregnancy after oxytocin (OT) and atosiban (A) administration, in vitro (n = 6 per treatment). OT treatment increased the level of *Aqp2* mRNA, but this effect was blocked by atosiban pre-treatment. ns $P > 0.05$, $^{***}P < 0.001$ as compared with the 18-day pregnant uterus (18 days).

reabsorption of luminal fluid (Lindsay and Murphy, 2006). *Aqp5* is also expressed in a distinct uterine cell type during the peri-implantation period, when the organ displays edema and hyperemia (Richard et al., 2003). Our study demonstrated the presence of *Aqp1*, *2*, *3*, *5*, *8*, and *9* in the

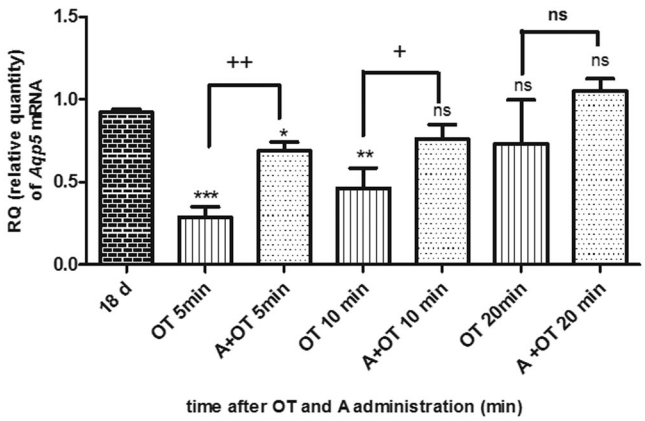

Figure 5. Changes in the expression of *Aqp5* mRNA 5, 10, and 20 min after pre-treatment with oxytocin (OT) and atosiban (A), in vitro (n = 6 per time point). ns $P > 0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to the 18-day pregnant uterus (18 days). ns $P > 0.05$, $^{+}P < 0.05$, $^{++}P < 0.01$, as compared between different treatments at the same time points.

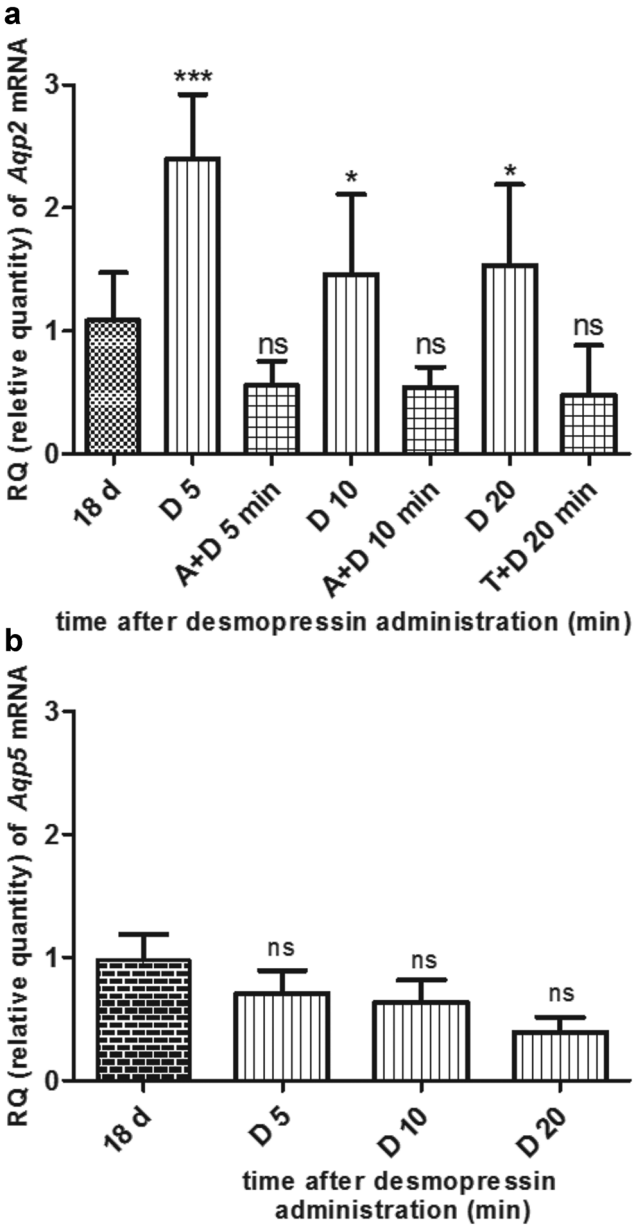

Figure 6. Changes in the expression of *Aqp2* (**a**) and *Aqp5* (**b**) mRNA after pre-treatment with desmopressin (D) and atosiban (A), in vitro (n = 6 per time point). The expressions of *Aqp2* increased, but that of *Aqp5* mRNA was unchanged after desmopressin administration. ns $P > 0.05$, $^{*}P < 0.05$, $^{***}P < 0.001$ as compared with the 18-day pregnant uterus (18 days).

late-pregnancy rat uterus, with a prevalent accumulation of the *Aqp5* subtype near parturition. We detected *Aqp5* transcript and protein, both of which were dramatically down-regulated on the last day of pregnancy. In the blood of late-pregnancy rats, however, the levels of detectable of *Aqp5* mRNA and protein exhibited a reciprocal pattern relative to that in the late-pregnancy uterus. Although,

we currently have no explanation for this phenomenon, the characteristic increase in *Aqp5* expression in the blood on the last day of pregnancy provides a valuable diagnostic marker for predicting the time of delivery. Such an application is consistent with the clinical use of a number of proteins (ADAM-12, activin-A, PAPP-A, inflammatory cytokines, and etcetera) to detect ectopic pregnancy and pre-term birth by means of routine blood tests (Rausch and Barnhart, 2012; Bastek and Elovitz, 2013).

Sexual hormones (progesterone and estrogen) regulate the expression of *AQPs* (Kim et al., 2009; Ji et al., 2013; Zou et al., 2013), while the anti-diuretic effect of vasopressin regulates the expression of *AQP2* (Marples et al., 1998). OT differs from vasopressin in only two amino acids. Both form a cyclic structure via a disulfide linkage between the cysteines in positions 1 and 6 (Terashima et al., 1999). OT is one of the main uterine-contraction-regulating hormones in late pregnancy. Near the time of parturition, there is a significant increase in *OT* mRNA synthesis, accompanied by an increase in the local concentration of OT peptide (Mitchell et al., 1997). OT stimulates its own receptor, but also cross-reacts with the vasopressin V_{1a} receptor (Akerlund et al., 1999). OT is also known to possess anti-diuretic properties, and the use of OT to induce labor is associated with water retention and hyponatremia (Li et al., 2008). By analogy, then it is not surprising that vasopressin can influence uterine function via its V_{1a} receptors and, to some extent, OT receptors. These similarities between OT and vasopressin suggest that OT should influence the function of AQPs in the pregnant uterus.

To test the possibility that OT directly affects AQP function during pregnancy, we investigated the myometrial expression of *Aqp5* and *Aqp2* mRNA after OT treatment and in the presence of the OT antagonist atosiban. Our in vitro studies were carried out on Day 18 of pregnancy because the expressions of *Aqp5* and *Aqp2* mRNAs were most pronounced at this time. OT treatment increased *Aqp2* mRNA levels in the uterus, which is similar to effects of OT in the kidney (Li et al., 2008; Cheng et al., 2009). OT treatment also specifically reduced the expression of *Aqp5* mRNA in the pregnancy-Day-18 uterus through its own receptors since the OT receptor antagonist atosiban inhibited the effects of OT on the expressions of *Aqp5* and *Aqp2* mRNA. In contrast, desmopressin, a synthetic analog of vasopressin, did not influence the expression of *Aqp5* mRNA, but did increase *Apq2* mRNA in the uterus. This effect was atosiban-sensitive, which means that the uterine effect of desmopressin is mediated mainly through OT receptors. Although, atosiban also exerts an antagonist effect on the V2 receptor, this receptor is not expressed in the myometrium (Manning et al., 1995).

In summary, our experiments have furnished basic information about the dynamic expression of *Aqps* in the late-pregnancy uterus. In light of our results, we presume that AQP5, regulated by OT, may play a role in the regulation of delivery. Furthermore, the detection of this protein in the blood may be of diagnostic value as a predictor of parturition timing.

MATERIALS AND METHODS

Housing and Handling of the Animals

Animals were treated in accordance with the European Communities Council Directives (86/609/ECC) and the Hungarian Act for the Protection of Animals in Research (XXVIII.tv.32.Ï). All experiments involving animal subjects were carried out with the approval of the Hungarian Ethical Committee for Animal Research (registration number: IV/198/2013). Sprague–Dawley rats (Charles-River Laboratories, Budapest, Hungary) were kept at $22 \pm 3^{\circ}C$ in a relative humidity of 30–70% with a light/dark cycle of 12/12 hr. The animals were maintained on a standard rodent pellet diet (Charles-River Laboratories) with tap water available ad libitum. They were sacrificed by CO_2 inhalation.

Mating of the Animals

Mature female (180–200 g) and male (240–260 g) Sprague–Dawley rats were mated in a special cage. A moveable metal door, controlled by a small electric motor that was turned on by a timer, separated the rooms for the male and female animals. Since, rats are usually active at night, the door was opened before dawn. Within 4–5 hr after a possible mating, vaginal smears were taken from the female rats to search for sperm using a microscope at a 1,200× magnification. When sperm were found or if a smear was not possible because of an existing vaginal sperm plug, the female rats were separated and marked as first-day pregnant animals.

Reverse-transcriptase-PCR Studies

Tissue isolation Rats (250–300 g) were anesthetized with sodium pentobarbital (1 g/kg, intra-peritoneal). Uterine tissues from pregnant animals (tissue between two implantations sites) and non-pregnant uteri were rapidly removed and placed in RNAlater Solution (Sigma-Aldrich, Budapest, Hungary). The tissues were frozen in liquid nitrogen and then stored at $-70^{\circ}C$ until the extraction of total RNA.

Blood collection On selected days of pregnancy (Days 18, 20, 21, and 22), rats were anesthetized with isoflurane, and blood samples were taken by cardiac puncture, collected in RNAprotect® Animal Blood Tubes (Qiagen, Biomarker Kft., Budapest, Hungary), and stored at $-20^{\circ}C$.

Total RNA preparation from tissue Total cellular RNA was isolated by extraction with guanidinium thiocyanate/acid–phenol/chloroform according to the procedure of Chomczynski and Sacchi (1987). After precipitation with isopropanol, the RNA was washed with 75% ethanol, and then resuspended in diethyl pyrocarbonate-treated water. Total RNA was isolated from 500 μl of whole rat blood through use of the RNeasy® Protect Animal Blood Kit (Qiagen, Biomarker Kft.).

TABLE 1. Assay IDs of the Applied Primers

TaqMan assays	Assay ID (Life Technologies, Hungary)
Aqp1	Rn00562834_m1
Aqp2	Rn00563755_m1
Aqp3	Rn00581754_m1
Aqp5	Rn00562837_m1
Aqp8	Rn00569732_m1
Aqp9	Rn00576331_m1
β-actin	Rn00667869_m1

RNA purity was assessed at an optical density of 260/280 nm with a BioSpec Nano (Shimadzu, Simkon Kft., Budapest, Hungary); all samples exhibited an absorbance ratio in the range 1.6–2.0. RNA quality and integrity were assessed by agarose gel electrophoresis.

Real-time quantitative reverse-transcriptase PCR Reverse transcription and amplification of the PCR products was performed using the TaqMan RNA-to-C_T^{TM} 1-Step Kit (Life Technologies, Budapest, Hungary) and an ABI StepOne Real-Time cycler. Reverse-transcriptase PCR amplifications were performed as follows: 48°C for 15 min and 95°C for 10 min, followed by 40 cycles at 95°C for 15 sec and 60°C for 1 min. The generation of specific PCR products was confirmed by melting curve analysis. Table 1 contains the assay IDs for the primers used. The amplification of β-actin (*Actb*) served as an internal control. All samples were run in triplicate. The fluorescence intensities of the probes were plotted against PCR cycle number. The amplification cycle displaying the first significant increase of the fluorescence signal was defined as the threshold cycle (C_T).

Western Blot Analysis

Twenty micrograms of sample protein per well were subjected to electrophoresis on 4–12% NuPAGE Bis–Tris Gel in XCell SureLock Mini-Cell Units (Life Technologies). Proteins were transferred from gels to nitrocellulose membranes using the iBlot Gel Transfer System (Life Technologies). The blots were incubated on a shaker with anti-AQP5 and anti-β-actin polyclonal antibodies (Santa Cruz Biotechnology, Inc., Dallas, TX, 1:200) in blocking buffer. Antibody binding was detected with the WesternBreeze Chromogenic Western blot immundetection kit (Life Technologies). Images were captured with the EDAS290 imaging system (Csertex Ltd., Budapest, Hungary), and the optical density of each immunoreactive band was determined with Kodak 1D Images analysis software. Optical densities were calculated as arbitrary units after local area background subtraction.

In Vitro Study

Uteri were removed from rats on Day 18 of pregnancy. Muscle rings 5-mm long were sliced from the uterine horns and mounted vertically in an organ bath containing 10 ml de Jongh solution (137 mM NaCl, 3 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 12 mM $NaHCO_3$, 4 mM NaH_2PO_4, and 6 mM glucose, pH 7.4). This organ bath was maintained at 37°C with carbogen (95% O_2, 5% CO_2) bubbled through it. After mounting, the rings were equilibrated for about 1 hr, with a solution change every 15 min, before experiments were undertaken. Uterine samples were incubated with OT (10^{-10} M), desmopressin (3.7×10^{-8} M), and atosiban (10^{-6} M). Samples were collected after 5, 10, and 20 min for reverse-transcriptase-PCR analysis.

Statistical Analyses

All experiments were carried out on at least six animals, repeated three times. All curve fittings, data calculations, and statistical analyses were performed with Prism 5.0 software (Graph Pad Software, Inc., San Diego, CA). Group comparisons were made by one-way ANOVA tests with the Tukey's post-tests.

ACKNOWLEDGMENT

We thank Ágnes Csiszár for her technical assistance in the experiments.

REFERENCES

Agre P, King LS, Yasui M, Guggino WB, Ottersen OP, Fujiyoshi Y, Engel A, Nielsen S. 2002. Aquaporin water channels––From atomic structure to clinical medicine. J Physiol 542:3–16.

Agre P, Kozono D. 2003. Aquaporin water channels: Molecular mechanisms for human diseases. FEBS Lett 555:72–78.

Akerlund M, Bossmar T, Brouard R, Kostrzewska A, Laudanski T, Lemancewicz A, Serradeil-Le Gal C, Steinwall M. 1999. Receptor binding of oxytocin and vasopressin antagonists and inhibitory effects on isolated myometrium from preterm and term pregnant women. Br J Obstet Gynaecol 106:1047–1053.

Bastek JA, Elovitz MA. 2013. The role and challenges of biomarkers in spontaneous preterm birth and preeclampsia. Fertil Steril 99:1117–1123.

Cheng CY, Chu JY, Chow BK. 2009. Vasopressin-independent mechanisms in controlling water homeostasis. J Mol Endocrinol 43:81–92.

Chomczynski P, Sacchi N. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. Anal Biochem 162:156–159.

He RH, Sheng JZ, Luo Q, Jin F, Wang B, Qian YL, Zhou CY, Sheng X, Huang HF. 2006. Aquaporin-2 expression in human endometrium correlates with serum ovarian steroid hormones. Life Sci 79:423–429.

Jablonski EM, McConnell NA, Hughes FM, Huet-Hudson Y. 2003. Estrogen regulation of aquaporins int he mouse uterus: Potential roles in uterine water movement. Biol Reprod 69: 1481–1487.

Ji YF, Chen LY, Xu KH, Yao JF, Shi YF, Shanguan XJ. 2013. Reduced expression of aquaporin 9 in tubal ectopic pregnancy. J Mol Histol 44:167–173.

Kim SO, Lee HS, Ahn K, Park KJ. 2009. Effect of estrogen deprivation on the expression of aquaporins and nitric oxide synthases in rat vagina. Sex Med 6:1579–1586.

Kobayashi K, Yasui M. 2010. Cellular and subcellular localization of aquaporins 1, 3, 8, and 9 in amniotic membranes during pregnancy in mice. Cell Tissue Res 342:307–316.

Li C, Wang W, Summer SN, Westfall TD, Brooks DP, Falk S, Schrier RW. 2008. Molecular mechanisms of antidiuretic effect of oxytocin. J Am Soc Nephrol 19:225–232.

Lindsay LA, Murphy CR. 2006. Redistribution of aquaporins 1 and 5 in the rat uterus is dependent on progesterone: A study with light and electron microscopy. Reproduction 131:369–378.

Lindsay LA, Murphy CR. 2007. Aquaporins are upregulated in glandular epithelium at the time of implantation in the rat. J Mol Histol 38:87–95.

Mann SE, Dvorak N, Gilbert H, Taylor RN. 2006. Steady-state levels of aquaporin 1 mRNA expression are increased in idiopathic polyhydramnios. Am J Obstet Gynecol 194:884–887.

Manning M, Miteva K, Pancheva S, Stoev S, Wo NC, Chan WY. 1995. Design and synthesis of highly selective in vitro and in vivo uterine receptor antagonists of oxytocin: Comparisons with Atosiban. Int J Pept Protein Res 46:244–252.

Marples D, Christensen BM, Frokiaer J, Knepper MA, Nielsen S. 1998. Dehydration reverses vasopressin antagonist-induced diuresis and aquaporin-2 downregulation in rats. Am J Physiol 275:F400–F409.

Mitchell BF, Fang X, Wong S. 1997. Metabolism of oxytocin in rat uterus and placenta in late gestation. Biol Reprod 57:807–812.

Nong YQ, Liu FH, Chen Y, Wang F. 2013. The expression and distribution of aquaporin 3 in mouse embryos before and after vitrification. J Assist Reprod Genet 30:601–606.

Prat C, Blanchon L, Borel V, Gallot D, Herbet A, Bouvier D, Marceau G, Sapin V. 2012. Ontogeny of aquaporins in human fetal membranes. Biol Reprod 86:48.

Preston GM, Carroll TP, Guggino WB, Agre P. 1992. Appearance of water channels in Xenopus oocytes expressing red cell CHIP28 protein. Science 256:385–387.

Rausch ME, Barnhart KT. 2012. Serum biomarkers for detecting ectopic pregnancy. Clin Obstet Gynecol 55:418–423.

Richard C, Gao J, Brown N, Reese J. 2003. Aquaporin water channel genes are differentially expressed and regulated by ovarian steroids during the periimplantation period in the mouse. Endocrinology 144:1533–1541.

Sha XY, Xiong ZF, Liu HS, Di XD, Ma TH. Maternal-fetal fluid balance and aquaporins: From molecule to physiology. Acta Pharmacol Sin 2011. 32:716–720.

Skowronski MT. 2010. Distribution and quantitative changes in amounts of AQP1, 5 and 9 in the pig uterus during the estrus cycle and early pregnancy. Reprod Biol Endocrinol 8:109.

Terashima Y, Kondo K, Oiso Y. 1999. Administration of oxytocin affects vasopressin V2 receptor and aquaporin-2 gene expression in the rat. Life Sci 64:1447–1453.

Xiong Y, Tan YJ, Xiong YM, Huang YT, Hu XL, Lu YC, Ye YH, Wang TT, Zhang D, Jin F, Huang HF, Sheng JZ. 2013. Expression of aquaporins in human embryos and potential role of AQP3 and AQP7 in preimplantation mouse embryo development. Cell Physiol Biochem 31:649–658.

Wilson JL, Miranda CA, Knepper MA. 2013. Vasopressin and the regulation of aquaporin-2. Clin Exp Nephrol 17:751–764.

Zardoya R. 2005. Phylogeny and evolution of the major intrinsic protein family. Biol Cell 97:397–414.

Zou LB, Shi S, Zhang RJ, Wang TT, Tan YJ, Zhang D, Fei XY, Ding GL, Gao Q, Chen C, Hu XL, Huang HF, Sheng JZ. 2013. Aquaporin-1 plays a crucial role in estrogen-induced tubulogenesis of vascular endothelial cells. J Clin Endocrinol Metab 98:E672–E682.

MRD