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AQP1 gene expression is upregulated by arginine vasopressin and cyclic AMP agonists in trophoblast cells

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ABSTRACT

Aquaporins (AQPs) are water channels that regulate water flow in many tissues. As AQP1 is a candidate to regulate placental fluid exchange, we sought to investigate the effect of arginine vasopressin (AVP) and cAMP agonists on AQP1 gene expression in first trimester-derived extravillous cytotrophoblasts (HTR-8/Svneo) and two highly proliferative carcinoma trophoblast-like cell lines but with a number of functional features of the syncytiotrophoblast namely; JAR and JEG-3 cells. Our data demonstrated that AVP (0.1 nM) significantly increased the expression of AQP1 mRNA at 10 h in HTR-8/SVneo and JEG-3 cells ($P<0.05$). Both SP-cAMP, a membrane-permeable and phosphodiesterase resistant cAMP, and forskolin, an adenylate cyclase stimulator significantly increased AQP1 mRNA expression in all cell lines after 2 h in a dose-dependent manner ($P<0.05$) with a parallel increase in protein expression. In the time course study, 5 μM of either SP-cAMP or forskolin significantly stimulated AQP1 mRNA expression after 2 h in HTR-8/SVneo cells and after 10 h in JAR and JEG-3 cells. AQP1 protein expression was highest after 20 h in both HTR-8/SVneo and JEG-3 cells ($P<0.05$). AVP-stimulated cAMP elevation was blocked in the presence of 9-(tetrahydro-2′-furyl) adenine (SQ22536) (100 μM), a cell-permeable adenylate cyclase inhibitor ($P<0.05$). These results indicate that in trophoblasts-like cells AQP1 gene expression is upregulated by both AVP and cAMP agonists. Furthermore, our data demonstrate that a cAMP-dependent pathway is responsible for the AVP effect on AQP1. Thus, modulation of AQP1 expression by maternal hormones may regulate invasion and fetal–placental-amnion water homeostasis during gestation.

Introduction

For the developing fetus, proper amniotic fluid water balance is critical to maintaining a full term pregnancy (Tomoda et al., 1987). The placenta has been shown to be an important gateway for water balance, affecting fetal plasma osmolarity (Wintour, 1998) and regulation of amniotic fluid volume (Magann et al., 2003). Movement of water across the placental surface occurs via a slow lipid diffusion pathway (Jansson and Illsley, 1993) or by water-filled channels (aquaporins) either formed as transient pores in the lipid membrane or through fixed protein mediated sites (Huang and Thompson, 1996). To date 13 aquaporins (AQPs) have been identified in mammals [AQP 0–12], and they play a critical role in controlling water flow particularly in specialized fluid-conducting organs such as kidney, lachrymal glands, lung and fetal membranes (Wintour, 1998; Liu et al., 2000, Ross and Brace, 2001; Magann et al., 2003). AQP gene expression has been seen in both placenta and fetal membranes across a diversity of species (Hasegawa et al., 1994; Ma et al., 1997; Johnston et al., 2000; Damiano et al., 2001; Wang et al., 2001; Mann et al., 2002; Wang et al., 2004; Liu et al., 2004; Wang et al., 2006); however their role in placental-amniotic-fetal water homeostasis has not been fully defined.

AQP1 is a 28-kDa protein, initially recognized as a water permeable membrane constituent (Denker et al., 1988). It was first found in red blood cells and renal tubules (Denker et al., 1988). Soon after, it was detected in other tissues including several secretory and resorptive epithelia and in continuous endothelia of capillaries (Nielsen et al., 1997). AQP1 was also located in the human, rat, and ovine placentas (Hasegawa et al., 1994; Umenishi et al., 1996). In human placenta, AQP1 was detected in the syncytiotrophoblast (Hasegawa et al., 1994) and in the fetal membranes (Mann et al., 2002), where it was suggested to play a role in transporting water between the fetus and amniotic cavity. These investigators speculated that idiopathic polyhydramnios may be associated with a deficiency of AQP1 in human fetal membranes (Mann et al., 2005). Our laboratory showed that amniotic fluid volume was negatively correlated with fetal membrane AQP1 and placental AQP1 and AQP9 expression, and positively correlated with placental AQP3 expression (Beall et al., 2007).

For some time it was believed that kidney AQP2 was the only AQP that was regulated by arginine vasopressin (AVP) (Fushimi et al., 1993; Nielsen et al., 1993). However, data from Patil et al. (1997) suggested that AQP1 may also be regulated by AVP in Xenopus oocytes. More recently AQP7 was found decreased in response to AVP (Gu et al., 2006). AVP has an effect on water metabolism of the amniotic

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membranes (Ervin et al., 1986). It was found that patients with polyhydramniosis (excess amnionic fluid) had the highest AVP concentration (Punnonen et al., 1987). In chronic twin–twin transfusion syndrome (TTTS), the amniotic concentrations of AVP were higher in the donor twin and lower in the recipient sac of the recipient baby which suggests that oligohydramnios (inadequate amniotic fluid) may occur as a consequence of AVP mediated reduction in fetal urine output (Bajoria et al., 2004). Oligohydramnios can be reduced and plasma osmolality stabilized leading to amniotic fluid volume increase by maternal 1-deamino-[8-D-arginine] vasopressin administration (Ross et al., 1996). It was suggested that the placenta may be permeable to small amounts of AVP or may itself produce this hormone (Punnonen et al., 1987). Therefore, we speculate that regulation of water permeability by AVP has potential relevance to active water transport not only in the amnion and fetal membranes but also in a variety of tissues that express AQP1 including the placenta.

Many hormones controlling water homeostasis including AVP exert their effects via cAMP (3′, 5′-cyclic adenosine monophosphate) by altering AQPs gene expression (DiGiovanni et al., 1994; Itoh et al., 2003; Wang et al., 2006). It was shown that the application of AVP to Xenopus oocytes injected with AQP1 cRNA increased the membrane permeability to water (Patil et al., 1997). Furthermore incubation with 8-bromo-cAMP or direct 8-bromo-cAMP injection into oocytes expressing AQP1 cRNA significantly increased membrane permeability to water, suggesting that stimulation of AQP1 activity by AVP involves a cAMP-dependent mechanism (Patil et al., 1997). Previous work in our laboratory demonstrated that AQP3 is stimulated by cAMP agonists in human amnion cultures (Wang et al., 2006) suggesting a role for this molecule in amniotic fluid homeostasis. Since the placenta may have a role in amniotic-fetal water homeostasis through AQP regulation, the modulation of these AQPs in the placenta may be influenced by the level of AVP or cAMP in this organ.

Several investigators used cell models that include primary isolates of villous trophoblasts (Genbacev et al., 1996), choriocarcinoma cell lines [BeWo, JAR and JEG-3 (Zygmunt et al., 1998; Mandl et al., 2002)] and SV40 transfected cell lines such as HTR-8/SVneo cells (Graham et al., 1993). These cell lines have extended life spans and their use avoids the variability of human placentas and pregnancies. In our study we used invasive first trimester extravillous HTR-8/Svneo, together with invasive trophoblast-like choriocarcinoma JAR and JEG-3 cells which possess functional features of the syncytiotrophoblast, to investigate the role of AQP1 in the placenta. These cells were compared for the level of expression of AQP1 in normal conditions and after hormonal treatments.

We hypothesized that AVP may have a regulatory role on placental AQPs; specifically placental AQP1 through cAMP signaling. To this end, we tested the effect of a 0.1 nM physiological concentration (Byron and Lucchesi, 2002) and 10 nM AVP (Han et al., 1993) to address the possibility that AVP could have a physiological role in regulating AQP1 in placental trophoblast cells. We also examined an AVP time course in JEG-3 cells. Since AVP acts through cAMP signaling pathway in other tissues (Patil et al., 1997) and AQP1 is regulated physiologically via cAMP-inducing forskolin (Wang et al., 2001), we examined increasing concentrations of SP-cAMP, a membrane-permeable and phosphodiesterase resistant cAMP, and forskolin, an adenylate cyclase stimulator on inducing AQP1 gene expression in a dose- and time-dependent manner. Furthermore, we assessed the specificity of AVP and cAMP agonists by using 9-(tetrahydro-2′-furyl) adenine (SQ22536) (100 μM), a cell-permeable adenylate cyclase inhibitor in the presence and absence of either AVP or a cAMP agonists.

Materials and methods

Cell lines and culture conditions

The HTR-8/SVneo cell line was a generous gift from Professor Charles H Graham, Queen's University, Kingston, ON, Canada. These cells were obtained from explant explant cultures of human first trimester placenta (8–10 week gestation) and were immortalized by transfection with a cDNA construct that encodes the simian virus 40 large T antigen (Graham et al., 1993). They exhibit phenotypic properties of extravillous cytotrophoblasts. The rapidly growing JAR cell line has growth characteristics of mono- and syncytiotrophoblasts (Greenwood et al., 1996) and the clonal transformed trophoblast JEG-3 cell line which displays a number of the functional features of syncytiotrophoblasts even though the cells are mononucleated and highly proliferative (Ringler and Strauss, 1990) were kindly donated by Professor Glenn D Braunstein, Cedars Sinai Medical Center, Los Angeles, CA, USA.

All the cell lines were maintained in a complete medium containing RPMI 1640 (Invitrogen Life Technologies, Inc., Grand Island, NY) medium supplemented with 5% heat inactivated fetal bovine serum (FBS; Invitrogen Life Technologies, Inc., Grand Island, NY) and 100 IU/ml of penicillin and 100 μg/ml of streptomycin (Invitrogen Life Technologies Inc., Grand Island, NY). At confluence, cells were detached from flasks using trypsin-EDTA mixture (Invitrogen Life Technologies Inc., Grand Island, NY) and seeded in a complete medium, at a density of 1×10^6 cells/well in 6-well dishes (Corning Inc., Wilkes Barre, PA) for 24 h. Cells were cultured in serum free and hormone-deprived RPMI for another 12 h before treatments.

Reagents

AVP (Sigma-Aldrich Corp., St. Louis, MO) was diluted in sterile double distilled water to a final concentration of 0.1 nM and 10 nM. SP-cAMP (Sigma-Aldrich Corp., St. Louis, MO) and forskolin (Sigma-Aldrich Corp., St. Louis, MO) stock solutions were diluted in sterile double distilled water and dimethlyl sufoxide (DMSO), respectively, to obtain a 2 mM stock solution. 9-(Tetrahydro-2′-furyl) adenine (SQ22536) (Calbiochem, La Jolla, CA) was diluted in sterile double distilled water to obtain a 500 μM stock solution.

Treatments

Effect of AVP on AQP1 gene expression

To test the effect of different concentrations of AVP on HTR-8/Svneo, JAR and JEG-3 cell lines, culture media containing 0.1 or 10 nM (final concentrations) of AVP were prepared by adding corresponding amounts of agonist to RPMI 1640 medium supplemented with 1% FBS. All cell lines were incubated for 10 h at 37 °C, 5% CO_2 followed by RNA extraction. For control cultures, cells were incubated in medium containing 1% FBS alone.

For AQP1 protein expression using either 0.1 nM or 10 nM of AVP for 20 h, we used HTR-8/Svneo and JEG-3 cell lines since preliminary data showed that JAR cells were unresponsive (data not shown). For control cultures, cells were incubated in medium containing 1% FBS alone.

Effect of cAMP agonists on AQP1 gene expression

To test the effect of different concentrations of cAMP agonists on the cell lines, culture medium containing 0.5, 5 or 50 μM (final concentrations) of either SP-cAMP or forskolin was prepared by adding corresponding amounts of agonist to RPMI 1640 medium supplemented with 1% FBS. Cells were then incubated for 2 h at 37 °C, 5% CO_2. For control cultures, cells were incubated in medium containing 1% FBS alone since we found no significant difference in the controls with or without DMSO that was used to make up stock solution of forskolin (results not shown).

For time course experiments, cells were incubated with 5 μM of either SP-cAMP or forskolin for 2, 10 and 20 h at 37 °C, 5% CO_2.

Mechanism of AVP action

To elucidate the possible regulation of AQP1 by AVP, JEG-3 cells were treated with 0.1 nM AVP in the presence of 100 μM SQ22536