KCNH1 potassium channels are expressed in cervical cytologies from pregnant patients and are regulated by progesterone


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Abstract

Potassium voltage-gated channel, subfamily H (eag-related), member 1 (KCNH1) potassium channels are potential tumour markers and cancer therapeutic targets and are up-regulated by oestrogens and human papilloma virus (HPV) oncogenes. However, the role of KCNH1 in normal tissues is poorly understood, and its expression in pregnancy is unknown. We wondered whether KCNH1 channels are expressed in cervical cells from pregnant patients and whether progesterone (P4) regulates KCNH1. The association with HPV was also investigated. KCNH1 protein expression was studied by immunocytochemistry in liquid-based cervical cytologies; 93 samples were obtained from pregnant patients at different trimesters, and 15 samples were obtained from non-pregnant women (controls). The presence of HPV was studied by PCR with direct sequencing and nested multiplex PCR. HeLa cervical cancer cells were transfected with human progesterone receptor-B (PR-B) and treated with P4. KCNH1 mRNA expression in these cultures was studied by real-time PCR. KCNH1 protein was detected in 100% of the pregnancy samples and in 26% of the controls. We found 18 pregnant patients infected with HPV and detected 14 types of HPV. There was no association between the percentage of cells expressing KCNH1 and either the presence or type of HPV. P4 induced KCNH1 mRNA and protein expression in cells transfected with human PR-B. No regulation of KCNH1 by P4 was observed in non-transfected cells. We show for the first time the expression of an ion channel during human pregnancy at different trimesters and KCNH1 regulation by P4 in human cells. These data raise a new research field for KCNH1 channels in human tissues.

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Introduction

Ion channels play major roles in human physiology, including neural transmission, heart muscle contraction, insulin secretion and cell proliferation. Alterations in either the expression or the activity of ion channels are associated with different diseases, including cardiac arrhythmias, epilepsy, cystic fibrosis, hyperinsulinemia and deafness (Ashcroft 2006). Voltage-gated potassium channels have been associated with several diseases and represent targets for many disorders, including cancer (Wulff et al. 2009). Ether à-go-go-1 (EAG1, KCNH1, Kv10.1) is a voltage-gated K⁺ channel that forms tetramers; each monomeric subunit has six putative transmembrane domains, with an intracellular C- and N-terminus. In the N-terminal region, it has a calmodulin binding site and a PAS domain (Per-Arnt-Sim), which has been suggested in other proteins to be an oxygen sensor, and in the C-terminal region, it has several domains including one cyclic nucleotide binding domain, a nuclear localisation signal and a calmodulin binding site (Schönherr et al. 2000, Bauer & Schwarz 2001, Downie et al. 2008, Wulff et al. 2009, Rodriguez-Rasgado et al. 2012).
KCNH1 has gained great interest in oncology and its expression has also been proposed as a cervical dysplasia marker (Ortiz et al. 2011). In addition, these channels are up-regulated by oestrogens and human papilloma virus (HPV) oncogenes (Díaz et al. 2009) and have been reported in almost 50% of patients taking oestrogens (Ortiz et al. 2011). Distribution of KCNH1 in normal human tissues is mainly restricted to the brain, myoblasts and adrenal gland and to the reproduction-associated organs placenta and testes (Occhiodoro et al. 1998, Pardo et al. 1999, Hemmerlein et al. 2006, Díaz et al. 2009). Nevertheless, the precise role of KCNH1 in normal tissues is nearly unknown.

During pregnancy, the cervix undergoes marked proliferation (Burger & Sherwood 1995, Cunningham et al. 2010), is under strong hormonal regulation and exhibits extensive remodelling, which includes softening, ripening, dilation/labour and post partum repair (Liggins 1978, Leppert 1995, Word et al. 2007, Timmons et al. 2010). The cervix is affected by increased cell proliferation and decreased apoptosis in epithelial and stromal cells; the peptide hormone relaxin, progesterone (P4) and oestrogens contribute to the process by promoting cell proliferation and/or inhibiting apoptosis (Zhao et al. 2001, Lee & Sherwood 2005, Lee et al. 2005). These hormonal effects are most pronounced during late pregnancy in rats, when the rate of cervical growth is higher (Zarrow & Yochim 1961, Zhao et al. 2001). The subtypes of oestrogen receptors ERα and ERβ are present in the human cervix and have been found to undergo dynamic changes in expression. During term pregnancy, the ERβ mRNA expression levels are increased, while the ERα mRNA expression levels remain unchanged. However, after parturition, ERβ and ERα expression decreases to the levels observed in non-pregnant women (Wang et al. 2001).

P4 is a very important steroid hormone in pregnancy because it promotes the morphological changes in the cervix and related tissues that help maintain pregnancy. P4 also inhibits smooth muscle contractions and the immune system, thus promoting an anti-inflammatory environment that helps maintain uterine quiescence (Hardy et al. 2006, Abeldazim et al. 2012, Byrns 2013). The predominant P4 receptor during human pregnancy is the B isoform (Goldman & Shalev 2007).

Some ion channels have been studied in the myometrium during pregnancy (Suzuki & Takimoto 2005, Xu et al. 2011). Aquaporins 3, 4, 5 and 8 are involved in fluid homoeostasis in pregnant and peri-partum cervices, thus facilitating water transport across the cervical epithelium (Anderson et al. 2006). However, despite the importance of proliferation and apoptosis in cervical epithelial cells, no reports exist on the expression of ion channels that are associated with proliferation/apoptosis in cervical cells during human pregnancy. KCNH1 channels have been associated with tumour cell proliferation, are regulated by oestrogens and are more commonly found in cervical cells from patients taking oestrogens (Pardo et al. 1999, Díaz et al. 2009, Ortiz et al. 2011). Therefore, to gain insight into the potential role of KCNH1 in normal human physiology, we tested whether KCNH1 channels are expressed in cervical cells under an oestrogen- and P4-enriched physiological environment such as human pregnancy.

**Subjects and methods**

**Biological samples**

Liquid-based cervical cytologies were obtained from 93 Mexican-Mestizo pregnant patients (14–37 years old) attending the General Hospital Dr Manuel Gea González in Mexico City, following local ethics considerations (protocol number 11-29-2010, approved by the Institutional Review Board). Patients were in the first, second or third trimester of pregnancy. Fifteen cervical samples from non-pregnant women (control patients with normal pap smears and no cervical pathology) attending the Instituto Nacional de Cancerología in Mexico City were also obtained, following local ethics considerations (protocol number 010/024/OMI/CB/622, approved by the Institutional Review Board). All patients provided written informed consent, and none of them had undergone any cervicovaginal treatment. Two samples per patient were taken. One sample was obtained using an endocervical brush and was used to study the presence and type of HPV. The other sample was taken using a broom-type cytobrush for liquid-based cervical cytology and was used to study KCNH1 expression.

**KCNH1 immunocytochemistry**

Specific anti-EAG1 MABs were kindly provided by Walter Stühmer and Luis Pardo (Max-Planck Institute for Experimental Medicine, Göttingen, Germany). This antibody has been extensively validated. The antibody has also been tested for specificity. It does not bind to Eag-related gene (Erg) channels and discriminates between Eag1 and Eag2 (Gómez-Varela et al. 2007). Using this antibody, we previously reported KCNH1 protein expression in Chinese hamster ovary cells transfected with KCNH1 (in contrast to no KCNH1 staining observed in non-transfected cells) and in human placenta and brain primary cultures from cervical cancer biopsies, cervical tissues from cervical intraepithelial neoplasia, cervical cancer cell lines and human cervical cytologies (Farias et al. 2004, Díaz et al. 2009, Ortiz et al. 2011). Normal keratinocytes (i.e. those not expressing KCNH1 mRNA) that were incubated with the primary anti-EAG1 antibody did not exhibit KCNH1 protein expression (Ortiz et al. 2011). KCNH1 immunocytochemistry was performed as described previously (Díaz et al. 2009, Ortiz et al. 2011). After standard treatments with anti-EAG1 antibody (1:100) and the mouse/rabbit secondary antibody (Biocare Medical, Concord, CA, USA), slides were washed followed by a 10-min incubation with peroxidase-linked polymer mouse/rabbit (Biocare Medical). Specific staining reactions were completed by incubating the slides in the presence of...
3,3′-diaminobenzidine in reaction buffer, which was observed as a brown staining. Sections were counterstained with haematoxylin (Dako, Glostrup, Denmark), washed with buffer and subsequently exposed to ethanol (Sigma–Aldrich), absolute alcohol (J T Baker, Phillipsburg, NJ, USA), xylene/absolute alcohol (1:1) and xylene (Sigma–Aldrich). Slides were mounted with Entellan resin (Merck) and observed using a Nikon Eclipse E50i microscope (Nikon, New York, NY, USA). Brown immunostaining revealed KCNH1 expression. Slides were blindly analysed by two pathologists. Images were obtained using a digital COOLPIX P5100 Nikon camera.

HeLa cervical cancer cells were purchased from ATCC, Manassas, VA, USA and transfected with progesterone receptor-B (PR-B), grown on glass coverslips and cultured in the presence of either P₄ (1 x 10⁻⁸ M) or ethanol (vehicle) for 48 h. Cells were processed as described earlier for KCNH1 immunocytochemistry. Quantification of immunostaining was performed using digital images of systematic, randomly selected fields. Five to seven fields of epithelial cells were measured using ImageJ software (NIH, Bethesda, MD, USA). The signal intensity was measured as pixels per cell nuclei area.

**HPV detection and typing**

Cervical samples were digested with 1 ml lysis buffer (10 mmol/l Tris–HCl, pH 8.0, and 1 mol/l EDTA (Gibco-BRL), pH 8.0, 0.5% SDS, 200 µg/ml proteinase K and 20 µg/ml RNase A) at 55 °C for 3 h. DNA was extracted with phenol/chloroform and precipitated with ethanol as described by Sambrook et al. (1989). To test the DNA suitability for PCR amplification, the DNA obtained was amplified for β-globin (PCO4/GH2O) under conditions described by Resnick et al. (1990). Samples were later submitted for HPV amplification using two sets of the following universal primers recognising distinct size fragments of the L1 gene: L1C1/L1C2.1/L1C2.2 and MY09/MY11 (van den Brule et al. 1990, Snijders et al. 1990, Yoshikawa et al. 1991). Amplification using MY09 and MY11 produced a conserved 450 bp fragment from the L1 gene, and amplification using the LC primers produced a 250 bp fragment of the L1 gene located upstream of the MY sequence. The following PCR protocol was used: 94 °C for 10 min, 38 cycles at a Tₘ of either 55 °C (for MY primers) or 49 °C (for LC primers) for 50 s and 72 °C for 7 min. DNA extracted from CaSkI and Hela HPV-containing cells (from ATCC) were used as controls. Mixtures without DNA were included as negative controls. HPV PCR products were electrophoresed on a 1.2% agarose gel (Gibco-BRL) and visualised with ethidium bromide staining. HPV typing was carried out using direct sequencing of PCR products with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The resulting sequences were analysed using the BLAST NCBI GenBank program (http://www.ncbi.nlm.nih.gov). Reagents were obtained from Sigma–Aldrich, unless otherwise indicated. Multiple HPV infections were analysed through nested multiplex PCR described by Sotlar et al. (2004), based on consensus and type-specific primers, amplifying a region of E6/E7 genes.

**Transient human PR transfection**

The expression vector for human PR (pLEN-hPRB) used to transfect HeLa cells was provided by Dr A J Cooney (Baylor College of Medicine). Cells were transfected using PolyFect (Qiagen, Inc.), as described previously (Diaz et al. 2009). The transfection of PR was confirmed using qRT-PCR. HeLa WT cells and PR-transfected cells were incubated for 24 h in the presence of increasing concentrations of P₄ (1 x 10⁻¹⁰–1 x 10⁻⁷ M) or vehicle (ethanol).

**Real-time PCR**

After exposing the cells to the described treatments, medium was aspirated and RNA was extracted using TRIzol reagent. cDNA was produced using 3 µg total RNA and the transcriptor RT system (Roche Diagnostics). Real-time PCR was performed using the LightCycler 2.0 from Roche, according to the following protocol: activation of Taq DNA polymerase and DNA denaturation at 95 °C for 10 min and 45 amplification cycles consisting of 10 s at 95 °C, 30 s at 60 °C and 1 s at 72 °C. The following primer sequences were used: PR, tca agc ttc aag tta gcc aag a, gac ttc gta gcc ctt cca aa; KCNH1, cct gga ggt gat cca aga tg, cca aac acg tct ctt cc. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control: aag cac atc gct gat cca c, gcc cta tcc gac cca atc c.

**Statistical analysis**

Statistical analysis was performed with the χ² test, Student’s t-test and one-way ANOVA using the SPSS statistical program. A P value <0.05 was considered significant.

**Results**

**KCNH1 expression in cervical cytologies from pregnant patients**

We first examined the expression of KCNH1 channels in cervical cytologies from pregnant women at different trimesters (Fig. 1). KCNH1 expression was observed in intermediate cells and superficial cells; however, the cells that were negative for KCNH1 were most commonly observed in superficial cells of the cervical layer. Previously obtained placental sections (Ortiz et al. 2011) were used as a positive control in which the expected KCNH1 expression was observed, whereas no staining emerged in the absence of the primary antibody (Fig. 1). We observed KCNH1 expression in all the samples from pregnant patients (Table 1). KCNH1 expression in normal cervical cytologies was reported in 27% of the patients (Ortiz et al. 2011). Accordingly, we found that 26.6% of the samples from non-pregnant women (i.e. control patients with pap smears that were negative for intraepithelial lesions) were positive for KCNH1 (Table 1).

Although all the samples from pregnant patients were positive for KCNH1, the percentage of positive cells from
each sample ranged from 5 to 90%. This finding was consistent among samples from different trimesters (Fig. 2). Most of the cervical cytologies from non-pregnant women were negative for KCNH1 expression, and none of them contained more than 66% of positive cells. By contrast, most of the samples from pregnant patients in the second and third trimesters displayed more than 67% of positive cells, with many samples exhibiting 100% of KCNH1-expressing cells (Fig. 2).

Because HPV oncogenes regulate KCNH1 channel expression, we studied the potential presence and, in the corresponding cases, the type of HPV in cervical cytologies from pregnant patients.

**HPV detection in cervical cytologies from pregnant patients**

To determine whether KCNH1 expression might be due to HPV infection, we studied the presence and type (i.e. low or high risk) of HPV in the cervical cytologies from pregnant patients. We identified HPV in 19% (18 of 93) of the samples, in accordance with previous reports (Hagensee et al. 1999, Medeiros et al. 2005, Castellsague et al. 2009). The HPV type was defined by direct sequencing of the PCR product and using the nested multiplex PCR method. We identified 14 types of HPV distributed in 18 HPV-positive samples, with eight samples displaying more than one HPV type (Fig. 3). We next determined whether the percentage of cells expressing KCNH1 might be associated with the type of HPV. We did not identify any correlation between the percentage of KCNH1-positive cells and either the presence or type of HPV (Fig. 3).

**P₄ regulates KCNH1 expression**

The lack of association between KCNH1 expression and the presence of HPV prompted us to study KCNH1 regulation by a very important hormone in human pregnancy, P₄. We previously demonstrated that oestrogens up-regulate KCNH1 mRNA expression in cervical cancer cells and that ERα is necessary for this regulation. We analysed KCNH1 mRNA and protein expression in the presence of P₄ in HeLa cervical cancer cells using real-time PCR and immunocytochemistry respectively. We used WT HeLa cells and HeLa cells over-expressing human PR-B. We did not observe the P₄-mediated regulation of KCNH1 expression in WT, non-transfected cells. By contrast, P₄ induced a significant dose-response increase in KCNH1 mRNA expression in cells transfected with human PR (Fig. 4A). We next tested

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**Figure 1** Detection of KCNH1 in cervical cytologies from pregnant patients. Individual examples are shown for the different trimesters of pregnancy: first trimester (2–12 weeks, n=13), second trimester (13–24 weeks, n=33) and third trimester (25–40 weeks, n=47). KCNH1 immunostaining was observed in the cytoplasm, nucleus and membrane, as previously reported for several cell types (Díaz et al. 2009, Ortiz et al. 2011). No staining was observed in cervical cells in the absence of the primary antibody (lower panel, left). Normal human placenta was used as a positive control for KCNH1 expression and displayed clear immunostaining (lower panel, right), as previously reported (Díaz et al. 2009, Ortiz et al. 2011). Magnification: upper panels, 200×; middle and lower panels, 400×.

**Table 1** KCNH1 in cervical cytologies from pregnant and non-pregnant patients.

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<th>KCNH1-positive samples (%)</th>
<th>Pregnant</th>
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<td>93/93 (100)</td>
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whether P4 also up-regulated channel protein expression in PR-B-transfected cells. KCNH1 protein expression was also increased in transfected cells upon P4 treatment (Fig. 4B, C, D, E and F).

**Discussion**

Here, we show the expression of an ion channel during human pregnancy at different trimesters. As previously reported, we found KCNH1 expression in 26% of non-pregnant patients (Ortiz et al. 2011). By contrast, KCNH1 expression was observed in 100% of the cervical samples from pregnant patients. The cervix undergoes marked proliferation during pregnancy and is under strong hormonal regulation (Burger & Sherwood 1995, Cunningham et al. 2010). KCNH1 channels have been associated with cell proliferation and are up-regulated by oestrogens (Pardo et al. 1999, Farias et al. 2004, Hemmerlein et al. 2006, Ousingsawat et al. 2007, Díaz et al. 2009, Asher et al. 2010). KCNH1 has been suggested to function as a tumour marker for different types of malignancies, including cervical cancer (Pardo et al. 1999, Farias et al. 2004, Hemmerlein et al. 2006). The expression of KCNH1 in normal cervical cytologies (negative pap smears) has been observed in 27% of the patients, whereas it is found in 67% of patients with low-grade cervical intraepithelial lesions and in 92% of patients with high-grade cervical intraepithelial lesions (Ortiz et al. 2011). In addition, KCNH1 has been reported in almost 50% of patients taking oestrogens (Ortiz et al. 2011). The oestrogenic regulation of KCNH1 channels has also been demonstrated in HeLa cervical cancer with oestradiol (Díaz et al. 2009). However, the role of KCNH1 channels in human placenta and testes remains unknown.

One of the most striking features of KCNH1 channels is its oncogenic potential and association with cancer (Pardo et al. 1999, Farias et al. 2004, Hemmerlein et al. 2006, Ousingsawat et al. 2007, Díaz et al. 2009, Asher et al. 2010). KCNH1 has been suggested to function as a tumour marker for different types of malignancies, including cervical cancer (Pardo et al. 1999, Farias et al. 2004, Hemmerlein et al. 2006). The expression of KCNH1 in normal cervical cytologies (negative pap smears) has been observed in 27% of the patients, whereas it is found in 67% of patients with low-grade cervical intraepithelial lesions and in 92% of patients with high-grade cervical intraepithelial lesions (Ortiz et al. 2011). In addition, KCNH1 has been reported in almost 50% of patients taking oestrogens (Ortiz et al. 2011). The oestrogenic regulation of KCNH1 channels has also been demonstrated in HeLa cervical cancer

**Figure 2** Pregnancy cervical cytologies exhibit a high percentage of KCNH1-positive cells. The percentage of KCNH1-positive cells was counted, and each sample was grouped into one of the following four levels of KCNH1 expression: No KCNH1 expression (0–4%), 5–33, 34–66, and 67–100% of positive cells.

![Figure 2](https://www.reproduction-online.org)

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**Figure 3** KCNH1 expression is not associated with HPV infection in cervical samples from pregnant patients. We found HPV infection in 18 patients, and we found 14 different HPV types (A). Co-infections with more than one HPV type were observed in eight patients. The percentage of cells expressing KCNH1 in each sample was not associated with either the presence or type of HPV detected (B) (P=0.715).

![Figure 3](https://www.reproduction-online.org)
Such regulation is strongly associated with the presence of ERα (Díaz et al. 2009). Thus, KCNH1 channels have also been proposed as cervical dysplasia markers and potential risk indicators of the disease. Nevertheless, the role of KCNH1 channels in normal human cervices is elusive.

Cervical epithelial proliferation decreases while apoptosis increases in post partum rats, with a concomitant decrease in ERα after delivery. The KCNH1 expression observed in 100% of the samples from pregnant patients might be associated with epithelial proliferation, hormone levels and the presence of ERα and PR-B. KCNH1 functions to maintain the homeostasis of oxygen in tumours by increasing VEGF and HIF-1 expression (Downie et al. 2008). During pregnancy, the cervix undergoes vascular changes to increase irrigation of the tissue; thus, KCNH1 might have a role in the regulation of cervical vascular homeostasis during pregnancy.

It is worth mentioning that the role of the KCNH1-mediated current in normal tissues is poorly understood. KCNH1 channel activity has been proposed to provide the hyperpolarising current required in myoblasts just before cell fusion (Occhiodoro et al. 1998). A nuclear localisation sequence is present in KCNH1 and potassium currents resembling Kcnh1 channel activity blocked by astemizole have been reported in the nuclear membrane (Chen et al. 2011). However, the potential role of KCNH1 in the nucleus remains elusive. In normal tissues, KCNH1 is mainly expressed in the brain (Pardo et al. 1999, Hemmerlein et al. 2006). Interestingly, Kcnh1 knockout mice have been produced, but the animals did not show any major abnormality at the CNS level (Ufartes et al. 2013). It is also known that ion channels have several non-conducting functions including interactions with other proteins like enzymes or cytoskeleton proteins (Kaczmarek 2006). Actually, non-conducting KCNH1 potassium channels are still able to induce tumour formation (Downie et al. 2008).

Definitely, it would be very interesting to study Kcnh1 expression during pregnancy in the Kcnh1 knockout model to gain insights into the potential role of KCNH1 channels in the cervix during pregnancy.

Because KCNH1 expression is up-regulated by HPV oncogenes (Diaz et al. 2009), we tested whether this variation might be due in part to HPV infection. We did not observe an association between KCNH1 expression and either the presence or type of HPV. In the case of non-pregnant women, probably it was only an early HPV infection that did not affect Kcnh1 expression, as it is known that persistent or recurrent HPV infections are needed to modify the cellular phenotype. It will be important to study Kcnh1 expression in cervical pap smears from patients who have been infected several times with HPV. On the other hand, because all the pregnant patients were positive for Kcnh1, perhaps KCNH1 regulation by hormones is stronger than the

Figure 4 KCNH1 expression is regulated by progesterone. (A) KCNH1 mRNA expression is significantly increased after P4 treatment in PR-B-transfected cells. Relative KCNH1 mRNA levels were obtained by normalising against GAPDH mRNA expression. Vehicle values were set to 1. Data are expressed as the mean±s.d. of triplicates from the same experiment. The graph is representative of two independent experiments. *P<0.05 vs control. (B, C, D, E and F) KCNH1 protein expression (brown immunostaining) is enhanced by P4. HeLa cells transfected with human PR-B were either treated with vehicle (B and D) or incubated in the presence of progesterone (1×10^{-8} M) for 48 h (C and E). Densitometric analysis showed a significant increase in KCNH1 protein expression in cells treated with progesterone (F). *P<0.05 vs vehicle and medium. Approximately 120 cells were analysed in each group. Magnification: (B and C), 200×; (D and E), 400×.
Cervical remodelling during pregnancy depends on P4 cells. By contrast, P4 increased KCNH1 expression in cervical cells of pregnant women is associated with hormonal regulation rather than HPV infection. We also found that the number of cells that express KCNH1 varies from 5 to 90% in pregnant women. While most of the cervical cytologies from non-pregnant women were negative for KCNH1 expression and none of them showed more than 66% of positive cells, most of the pregnancy samples from the second and third trimesters displayed more than 67% of KCNH1-positive cells with many samples exhibiting 100% of KCNH1-positive cells. We did not find significant differences between the percentage ranges of positive cells during different trimesters (Fig. 2). Nevertheless, this lack of significant difference might be due to either the number of samples in each trimester or the precise pregnancy time. KCNH1 expression in patients at the end of one trimester might be very similar to channel expression in the early stages of the following trimester. The pregnancy time of some patients was very close to the end of the trimester, and it was at the beginning of the second or third trimester in other cases. Thus, it will be very important to study KCNH1 expression during pregnancy in a more detailed manner. For example, dividing the patients in groups of 12, 24 or 36 weeks of pregnancy or using days of gestation instead of trimesters may give more insight into the regulation of KCNH1 expression during pregnancy. In addition, more quantitative studies would be useful. For example, quantitative analyses could be performed to evaluate KCNH1 mRNA or protein expression using real-time PCR or western blot techniques respectively. Doing so may lead to identification of a closer relationship between channel expression and gestation time. Hormone blood levels could also be quantified to assess a potential association with KCNH1 expression.

To our knowledge, this study is the first to show the regulation of KCNH1 mRNA and protein expression by P4. We did not observe hormonal regulation in HeLa WT cells. By contrast, P4 increased KCNH1 expression in cells transfected with human PR-B, strongly suggesting that this receptor is necessary for such regulation. P4 is essential in pregnancy, and high levels of this hormone are maintained during gestation (Hardy et al. 2006). Cervical remodelling during pregnancy depends on P4 because it can modulate the expression of some components of the extracellular matrix (ECM), thus inhibiting hyaluronate expression (Tanaka et al. 1994, 1997) and increasing collagenase, elastase and metalloproteinase levels (Andersson et al. 2008). Because the cervical tissue mechanical properties are related to the ECM (Myers et al. 2008) and because the ECM is the major component of cervical tissue (Leppert 1995), cervical softening is associated with decreased collagen organisation, which is characterised by increased collagen solubility and decreased collagen concentration. In pregnancy, collagen solubility increases early. In the third trimester, up to 90% of cervical collagen is soluble; thus, its concentration decreases ~50% compared with non-pregnant women (House et al. 2009, Myers et al. 2009). Human KCNH1-mediated potassium currents are decreased in cells grown on collagen (Toral et al. 2007). Thus, a plausible scenario is that the up-regulating effects of P4 on KCNH1 mRNA and protein expression are related to the inhibitory effect of P4 on collagen organisation/concentration.

There are several PR isoforms including PR-A (N-terminal truncated receptor), PR-B (full-length receptor) and PR-C (Evans 1988, Goldman & Shalev 2007). PR-B is the principal mediator of P4 actions and is the predominant form during pregnancy (Graham & Clarke 2002, Goldman & Shalev 2007). PR regulates genes that encode various ion pumps and channels (Connaghan et al. 2010). For example, P4 was shown to modulate the expression of TRPV4 channels via the PR (Jung et al. 2009). Taken together, these results suggest that P4 also regulates KCNH1 expression via PR-B, as shown in this study.

Rat KCNH1-mediated potassium currents are inhibited in Xenopus laevis oocytes after incubation with P4 (Brüggemann et al. 1997). Nevertheless, in X. laevis oocytes, P4 binds to a membrane PR, which is the only PR type identified in Xenopus (Sadler & Maller 1982). Here, we studied human KCNH1 channels and human PR-B, but more studies are needed to elucidate the mechanism of the P4-mediated regulation of KCNH1 expression.

Our results suggest a novel role for KCNH1 channels in cervical physiology during pregnancy, which is most likely associated with hormonal regulation and raises a new research field for KCNH1 channels in healthy human tissues.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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