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Research article

Cooperation of aquaporin 5 and the adrenergic system in the initiation of birth in rat model

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ABSTRACT

Aquaporins (AQPs) are involved in the process of implantation, regulate myometrial contractions and cervical ripening, and maintain appropriate placental functioning. The molecular mechanism of these functions is not fully understood. Our study aimed to investigate the physiological significance of AQP5 during pregnancy and to determine the cooperation between the adrenergic system and the AQP5 in uterine contraction in the late-pregnant rat uterus.

After administering AQP5 siRNA intraperitoneally to Sprague-Dawley rats, the length of the gestational period was determined and the changes in uterine contractions were measured in an isolated organ bath system. Pharmacological influence on AQP5 expression and uterine contraction was investigated by treatment with terbutaline (10 mg/kg, subcutaneously) and doxazosin (5 mg/kg, orally) *in vivo*; and mercuric chloride ($HgCl_2$), *in vitro*. Moreover, the levels of cAMP response element binding protein (CREB) were measured in the uterus by an ELISA kit.

The gestational period became shorter, AQP5 expression significantly decreased and rat uterus contraction increased after AQP5 siRNA treatment compared to the control. Treatment with terbutaline significantly increased AQP5 mRNA and protein expression after 30 min and continuously reduced it until 90 min, whereas doxazosin treatment did not significantly alter AQP5 expression. Treatment with the AQP5 antagonist $HgCl_2$ increased spontaneous uterus contraction and decreased norepinephrine-induced uterus contraction with decreasing AQP5 expression in pregnant rat uterus. Moreover, the tocolytic effect through the adrenergic system was amplified in the presence of an AQP5 antagonist, presumably via the changes in cAMP level.

In conclusion, our findings elucidate the collaborative role of aquaporin 5 (AQP5) and adrenergic systems in the regulation of uterine contractions in late-pregnant rats. Our findings suggest this may be a good starting point for developing a new tocolytic therapy.

1. Introduction

Spontaneous preterm birth, defined as the birth of a live infant prior to 37 completed weeks of gestation, is the primary contributor

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to infant mortality and morbidity globally [1]. In 2020, it is estimated that 13.4 million infants were born prematurely, representing more than one in ten births. Furthermore, in 2019, approximately 900,000 children succumbed to complications associated with preterm birth [2]. There are limited medications currently utilized in clinical practice for the treatment of preterm labor or the prevention of spontaneous preterm birth [3]. A 2022 Cochrane network meta-analysis on tocolytics concluded that all subclasses - including beta-adrenergic receptor mimetics, COX inhibitors, calcium channel blockers, magnesium sulfate, oxytocin receptor antagonists, and nitric oxide donors - are likely effective in delaying preterm labor by 48 h [4]. Among these agents, terbutaline, a β-agonist, interacts with β2-adrenergic receptors to decrease intracellular ionized calcium levels, thereby inhibiting the activation of myometrial contractile proteins, which constitutes its primary mechanism of action. The problem with these therapies is that they may delay delivery, but they do not permanently solve the problem of preterm birth. Based on the above, the major and important challenge for drug developers is to identify a new target or drug combination that can be used in long-lasting tocolytic therapy without maternal and fetal risks. To find new targets for therapy, we need to know more about the mechanisms of our induction of labor. Hopefully, AQP channels may be one of the promising targets in the regulation of the functioning of the uterine smooth muscle.

Aquaporins (AQPs) play a critical role in the molecular regulation of osmolarity, cell migration, junction adhesion, surface protein expression, cellular proliferation, extracellular matrix dynamics, stress response, energy metabolism, and the regulation of cellular water content [5]. Furthermore, AQP levels undergo modifications during the implantation process in both the uterus and fetal cells, and they play a role in regulating amniotic fluid. They also play an important role in fetal lung development; for example, in one study, mice with impaired lung development had lower AQP5 mRNA expression, and another study found low AQP5 expression in the lungs of sheep born preterm compared to control fetuses [6,7]. In addition, AQPs also seem to be very important for normal placental functions, and AQP5 has been localized to the chorionic areolae of the porcine placenta [8]. AQPs are involved in the control of myometrial contractions during pregnancy and cervical ripening [9].

Previously, we determined AQP5 dominance during gestation days 18–21 in the rat uterus, which was down-regulated considerably on day 22 of gestation. Furthermore, progesterone and progesterone analogs raised AQP5 expression, which was even more dominant than the estrogenic action, whereas oxytocin specifically decreased this specific type of water channel [10,11]. Based on these results, we found an inverse correlation between uterine contractions and the expression of AQP5 in the late-pregnant rat uterus. We hypothesize that low levels of AQP5 in the uterus may indirectly increase uterine contractility in late pregnancy.

Multiple signal transduction pathways are recognized to regulate the expression of aquaporins (AQPs) in mammals. We presume the cooperation between the adrenergic system and AQP5 in the pregnant uterus as a potential pathway. Consequently, our objective was to examine the physiological significance of AQP5 during pregnancy and assess the adrenergic system's impact on the expression of AQP5 and uterine contractions in rats.

2. Materials and methods

2.1. Housing and handling of the animals

Animals were treated following the European Communities Council Directive (2010/63/EU) and the Hungarian Act for the Protection of Animals in Research (Article 32 of Act XXVIII). All experiments involving animal subjects received approval from the National Scientific Ethical Committee on Animal Experimentation (registration number: IV/2767/2020). Sprague-Dawley rats (INNOVO Ltd., Gödöllő, Hungary) were maintained at a temperature of 22 ± 3 °C, with relative humidity ranging from 30 % to 70 %, and a light/dark cycle of 12 h each. The animals were provided with a standard rodent pellet diet (INNOVO Ltd., Gödöllő, Hungary) and had access to tap water ad libitum. Euthanasia was performed via CO_2 inhalation.

2.1.1. Mating of the animals

Mature female Sprague-Dawley rats, weighing between 180 and 200 g, and male rats, weighing between 240 and 260 g, were mated in a designated mating cage before dawn. Within 4 h following the mating, vaginal smears were collected, and a microscopic examination for sperm presence was conducted. If the examination yielded a positive result, the female rats were separated and classified as first-day pregnant subjects.

2.2. In vivo studies

- siRNA treatment

Animals (n = 6) were treated with AQP5 siRNA by intraperitoneal injection and physiological saline solution on day 21 (6 a.m.) of pregnancy. Ambion® In Vivo Pre-designed siRNA Product (1 mg/kg, siRNA ID: s1299246, Life Technologies, Hungary) was used for the treatment for AQP5 down-regulation. Transfection was made using in vivo-jetPEI® (Polyplus, VWR International Ltd., Hungray). After the treatment, the changes in the uterine contractions were investigated *in vitro* and the length of the gestational period *in vivo*. The beginning of labor was defined by the delivery of the first pup.

-Terbutaline and doxazosin treatment

Rats (n = 8 in each group) were treated with terbutaline (10 mg/kg, Sigma-Aldrich, Hungary) subcutaneously and doxazosin (5 mg/kg, Sigma-Aldrich, Hungary) orally on pregnancy day 21. The dosages of the medications were established by the available

literature [11,12]. Uterine tissues were collected 30, 60 and 90 min after treatment to investigate the changes in uterus contraction and AQP5 expression.

2.3. In vitro studies

2.3.1. Isolated organ bath study

The pregnant Sprague-Dawley rats were sacrificed by CO_2 inhalation. After the dissection of the two horns of the uteri, the fetuses were removed and the uteri were sliced into 5-mm-long rings. The smooth muscle tissues were positioned vertically within an organ bath containing 10 mL of de Jong solution, which has the following composition in millimoles per liter (mM): 137 NaCl, 3 KCl, 1 CaCl2, 1 MgCl2, 12 NaHCO3, 4 NaH2PO4, and 6 glucose, with a pH of 7.4. The temperature of the organ bath was maintained at 37 °C, and carbogen (composed of 95 % O2 and 5 % CO2) was continuously bubbled through the chambers.

-In vitro contractility studies

After setting the initial tension to 1.5 g, the uterus samples were allowed to equilibrate for 60 min with a buffer change every 15 min. The contractions of the 22-day myometrial rings were measured using a gauge transducer (SG-02; MDE Ltd., Budapest, Hungary) and recorded with a SPEL Advanced ISOSYS Data Acquisition System (MDE Ltd., Budapest, Hungary). In the siRNA experiments, spontaneous contractions were measured for 30 min, then rhythmic contractions were elicited with 25 mM KCl for 30 min. The contraction response of the control uterine samples (siRNA nontreated) to KCl was also measured.

In the $HgCl_2$ experiments, contractions were elicited with 10^{-5} M norepinephrine (NE) (Merck Ltd, Hungary) for 10 min without or with the presence of 10^{-5} M propranolol (Merck Ltd, Hungary) to avoid the β-adrenergic actions of NE. When equilibrated contractions were measured in both groups, the rings were washed 3 times, then $HgCl_2$ (100 μM) was administered into each chamber. After 15 min, the rings were washed 3 times again and allowed to recover for 30 min. Subsequently, the uterine tissues were contracted with five concentrations of NE (10^{-11}-10^{-7} M) in the presence of terbutaline (10^{-7} M) or doxazosine (10^{-7}) for 10 min. The control measurements were carried out in the same manner without $HgCl_2$ treatment.

The areas under the curves (AUC) were assessed and statistically analyzed using the Prism version 5.01 software (GraphPad Software, San Diego, CA).

2.4. RT-PCR studies

Tissue isolation. Pregnant uterus tissues were collected (trimmed free of fat, placental and adjacent tissue) and placed in RNAlater Solution (Sigma-Aldrich, Hungary), then stored at −75 °C until the extraction of total RNA.

Total RNA preparation from tissue. Total cellular RNA was isolated through extraction using guanidinium thiocyanate-acid-phenol-chloroform, following the methodology established by Chomczynski and Sacchi [13]. Subsequent to precipitation with isopropanol, the RNA was subjected to a washing step with 75 % ethanol and was then resuspended in diethylpyrocarbonate-treated water. The purity of the RNA was evaluated by measuring the optical density at 260/280 nm using a BioSpec Nano spectrophotometer (Shimadzu, Japan); all samples demonstrated an absorbance ratio ranging from 1.6 to 2.0. The quality and integrity of the RNA were further assessed through agarose gel electrophoresis.

Real-time quantitative reverse transcription-PCR (RT-PCR). Reverse transcription and amplification of the PCR products were performed by using the TaqMan RNA-to-C_T-Step One Kit (Thermo Fisher Scientific, Hungary) and an ABI StepOne Real-Time cycler. Reverse-transcriptase PCR amplifications were performed as follows: at 48 °C for 15 min and at 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and at 60 °C for 1 min. The generation of specific PCR products was confirmed by melting curve analysis. The following primers were used: assay ID Rn00562837_m1 for the *Aqp5* water channel and Rn00667869_m1 for *β-actin* as endogenous control (Thermo Fisher Scientific, Hungary). All samples were analyzed in triplicate. The probes' fluorescence intensities were plotted against the number of PCR cycles. The amplification cycle at which the first significant increase in the fluorescence signal was observed was designated as the threshold cycle (CT).

2.5. Western blot analysis

A total of 25 μg of protein per well was subjected to electrophoresis utilizing a 4–12 % NuPAGE Bis-Tris Gel within XCell SureLock Mini-Cell Units (Thermo Fisher Scientific, Hungary). The proteins were subsequently transferred from the gels to nitrocellulose membranes employing the iBlot Gel Transfer System (Thermo Fisher Scientific, Hungary). Antibody binding was detected using the WesternBreeze Chromogenic Western Blot Immunodetection Kit (Thermo Fisher Scientific, Hungary). The blots were incubated on a shaker with AQP5 (catalog number AB-15858, dilution 1:200, Sigma-Aldrich) and beta-actin (catalog number bs-0061R, dilution 1:200, Bioss Antibody) polyclonal antibodies in a blocking buffer. Images were captured utilizing the EDAS290 imaging system (Kodak Ltd., German), and the optical density of each immunoreactive band was quantified using Kodak 1D Image Analysis Software. Optical densities were calculated as arbitrary units following the subtraction of the local background from the area of interest.

2.6. CREB assay

Changes in the CREB concentration were determined to clarify the molecular relationship through cAMP between AQP5 and

adrenergic receptors. Following the manufacturers' recommendations, CREB levels in the uterus (ER0865, Fine Test, Wuhan Fine Biotech Co., Ltd.) were measured using rat ELISA Kits. This kit was developed utilizing sandwich enzyme-linked immunosorbent assay (ELISA) technology. The detecting antibody was the anti-CREB antibody coupled with biotin. Optical density values were measured utilizing a SPECTROStar Nano microplate spectrophotometer at a wavelength of 450 nm (BMG Labtech, Germany).

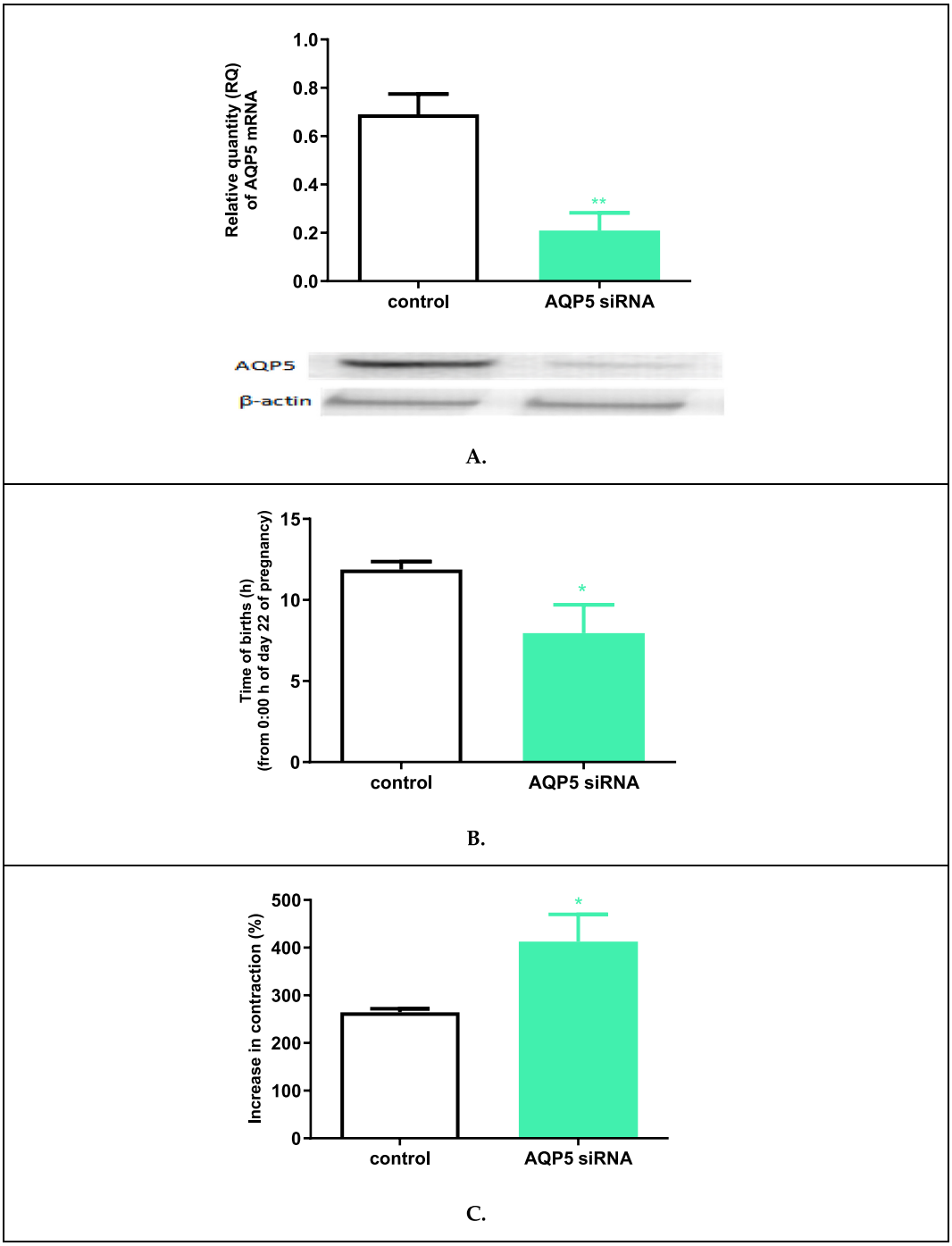

Fig. 1. AQP5 mRNA and protein expression (**A,** supplement1) in the uterus after siRNA treatment. Changes in the time of birth (**B**) and *in vitro* uterus contraction (**C**) after AQP5 siRNA treatment. *$p < 0.05$ and **$p < 0.01$ compared to the control.

2.7. Statistical analysis

In the statistical analysis, we systematically summarized our data to facilitate analysis and draw conclusions. Statistical analyses were conducted using Prism 10.2.1 software (GraphPad Software Inc., San Diego, CA, USA). All data were assessed using either a one-way ANOVA test (with Dunnett's post hoc test) or an unpaired *t*-test, and results are presented as the mean ± standard error of the mean (SEM). A significance level of $p < 0.05$ was established.

3. Results

3.1. siRNA studies

Following AQP5 siRNA treatment, AQP5 mRNA and protein expressions were significantly decreased in the uteri of the treated pregnant rats (Fig. 1A and B), in comparison to the control group, which was administered a physiological saline solution, indicating that downregulation of AQP5 channels occurred. The control animals gave birth on the morning of gestation day 22 (11.88 ± 0.4848 h), in contrast, the animals in the AQP5 siRNA-treated group had a shorter gestation period (7.95 ± 1.758 h), giving birth earlier than

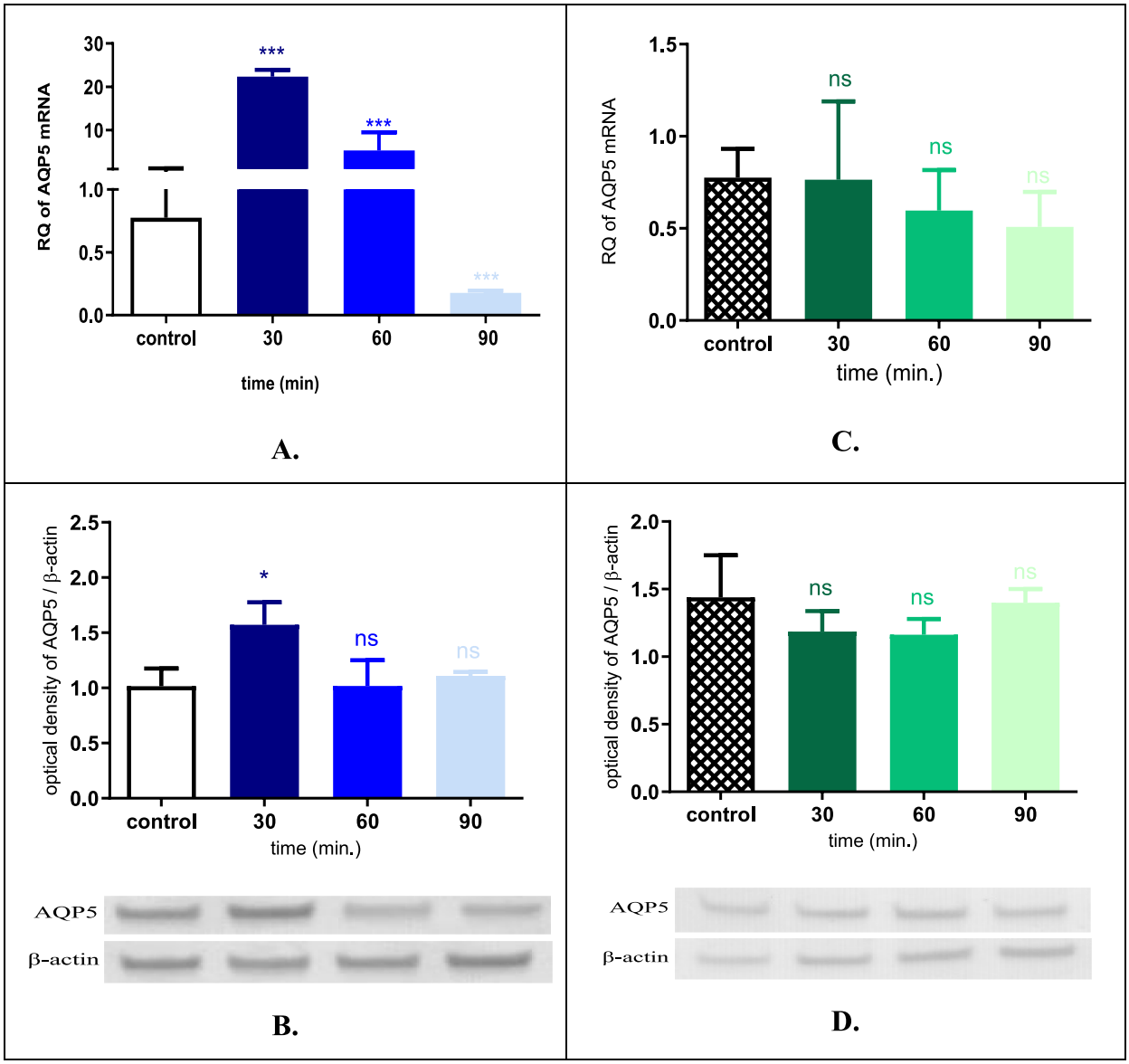

Fig. 2. Changes in AQP5 mRNA (A) and protein expression (supplement1) after terbutalin (B) and doxazosin treatment (C, D) in pregnant rat uterus. ns > 0.05; *p < 0.05, ***p < 0.001; compared to the control uterus sample. (The Western blot pictures are attached as a supplement file 1.).

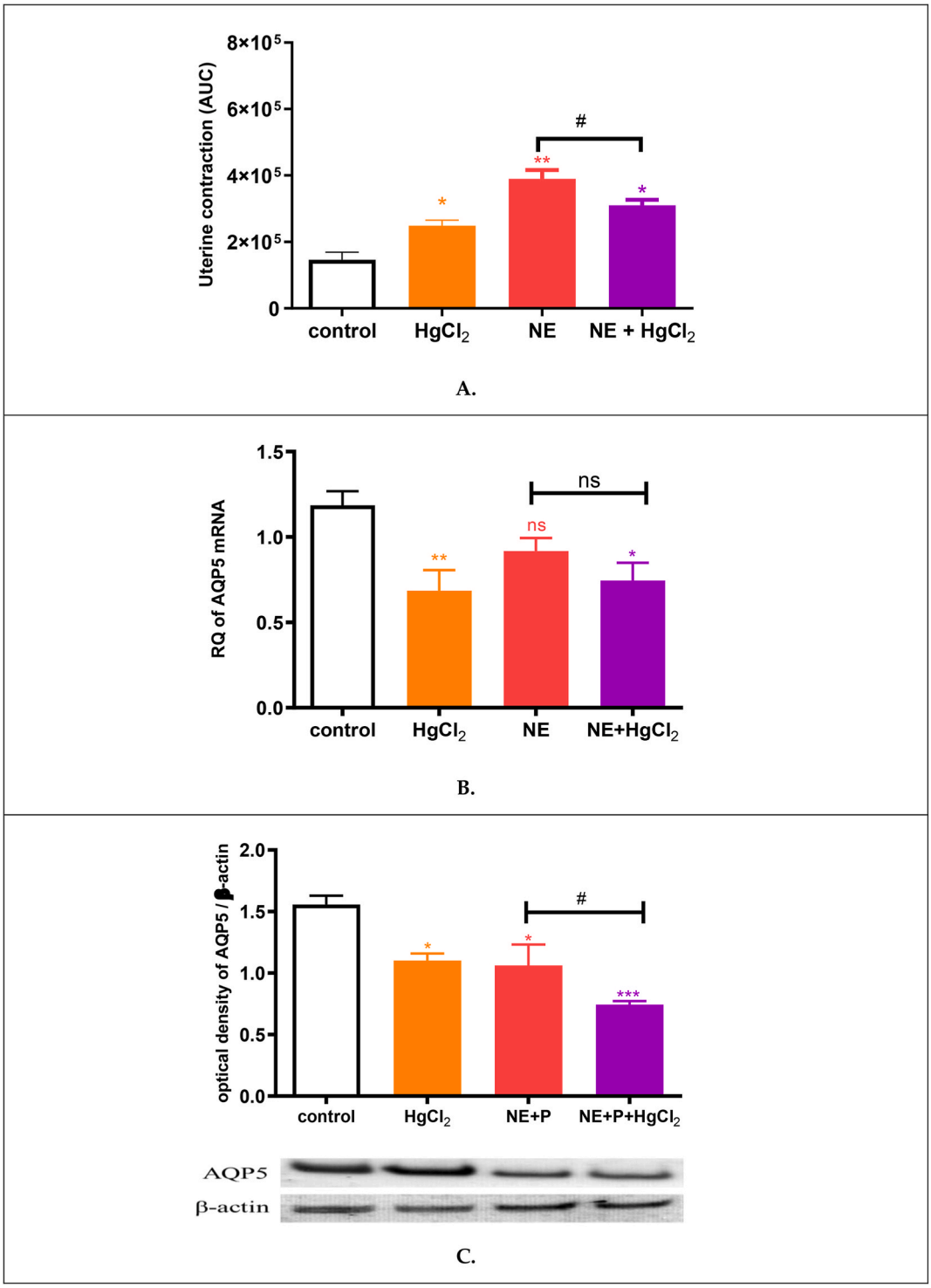

Fig. 3. Changes in the area under the curve (AUC) values of uterus smooth contraction (**A**), mRNA (**B**), and protein expression *(supplement2)* of AQP5 (**C**) of spontaneous and NE-induced uterine contractions before and after $HgCl_2$ treatment. ns $p > 0.05$, *$p < 0.05$, **$p < 0.01$ compared to the control. #$p < 0.05$ compared to the NE-treated uterus. (The Western blot pictures are attached as a supplement file 2.).

the control pregnant rats. The treatment of rats with AQP5 siRNA significantly increased (difference: 148.2 ± 57.56 %) the KCl-evoked *in vitro* uterine contractions (Fig. 1C) compared to the control animals.

3.2. Terbutaline and doxazosin treatments

The AQP5 mRNA expression was significantly increased in the 30th and 60th minutes after terbutaline treatment and significantly decreased in the 90th minute in the uterus samples (Fig. 2A). The changes in the AQP5 protein level followed the mRNA expression with the maximum in the 30th minute (Fig. 2B).

The AQP5 mRNA and protein expression did not change significantly after doxazosin treatment (Fig. 2 C, D).

3.3. In vitro $HgCl_2$ studies

The AUC values of spontaneous contractions were enhanced by $HgCl_2$ treatment in an *in vitro* isolated organ bath study of a 22-day-old pregnant uterus, which means that the uterine contractile activity was increased. In the other group, the norepinephrine (NE)-induced contractions significantly decreased after $HgCl_2$ treatment (Fig. 3A).

AQP5 mRNA (Fig. 3B) expressions significantly decreased in the $HgCl_2$-treated groups compared to the control. Protein expression significantly decreased by $HgCl_2$ in the presence of NE compared to the control uterus (Fig. 3C).

NE elicited a concentration-dependent uterine contraction, which was reduced by $HgCl_2$ treatment. In the presence of terbutaline, the concentration-response curve of NE was shifted down, representing a concentration-dependent reduction in the relaxing effect. The $HgCl_2$ treatment ceased this concentration dependency of the relaxing action of NE in the presence of terbutaline (Fig. 4A, Table 1). Propranolol did not modify the stimulating effect of NE, but doxazosin shifted it down. In the presence of propranolol, the $HgCl_2$ treatment turned the NE effect to relaxation, which was not modified in the presence of doxazosin (Fig. 4B–Table 2).

3.4. Results of CREB assay

We examined cAMP response element binding protein (CREB) levels in the treatment combination of norepinephrine (NE), $HgCl_2$,

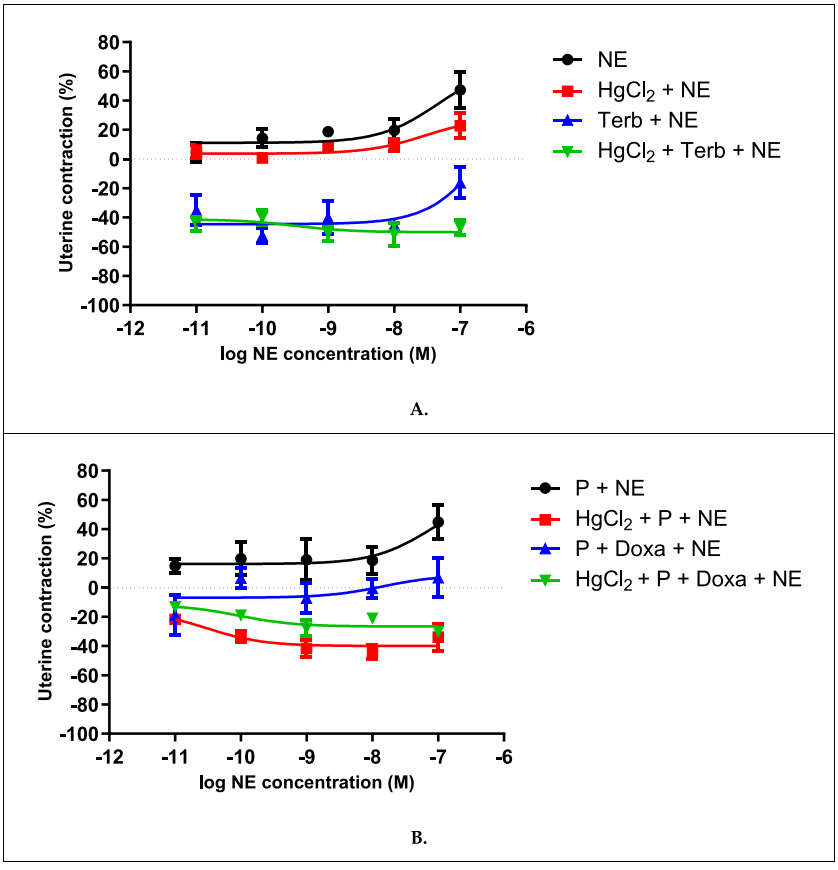

Fig. 4. Dose-dependent effect of norepinephrine (NE, 10^{-11} – 10^{-7} M) on pregnant uterine contractions (day 22) alone or in combination with $HgCl_2$, terbutaline (Terb) 10^{-7} M, propranolol (P) and doxazosin (Doxa) 10^{-7} M.

Table 1
Changes in norepinephrine EC_{50} and E_{max} values without or after $HgCl_2$ treatment in the presence of terbutaline or alone.

Substance	EC_{50} (M)	E_{max} (%)
NE	$4.4 \pm 1.1 \times 10^{-8}$	62.9 ± 11.1
NE + T	$4.9 \pm 1.4 \times 10^{-7}$*	63.0 ± 9.7
NE + $HgCl_2$	$2.8 \pm 0.7 \times 10^{-8}$	28.1 ± 6.4**
NE + $HgCl_2$ + T	$3.1 \pm 1.8 \times 10^{-10}$***	-50.8 ± 8.3***

NE: norepinephrine (10^{-11}-10^{-7} M), T: terbutaline (10^{-7} M), EC_{50}: half maximal effective concentration, E_{max}: maximal contracting effect (a negative sign means relaxation), *p < 0.05, **p < 0.01, ***p < 0.001.

Table 2
Changes in norepinephrine EC_{50} and E_{max} values without or after $HgCl_2$ treatment in the presence of propranolol or propranolol-doxazosin combination.

Substance	EC_{50} (M)	E_{max} (%)
NE + P	$7.5 \pm 1.9 \times 10^{-8}$	63.0 ± 15.6
NE + P + Doxa	$1.5 \pm 0.6 \times 10^{-8}$*	8.6 ± 3.1**
NE + P + $HgCl_2$	$3.0 \pm 1.2 \times 10^{-11}$***	-40.0 ± 8.1***
NE + P + $HgCl_2$ + Doxa	$9.1 \pm 2.7 \times 10^{-11}$***	-26.7 ± 9.5***

NE: norepinephrine (10^{-11}-10^{-7} M), P: propranolol (10^{-7} M), Doxa: doxazosin (10^{-7} M), EC_{50}: half maximal effective concentration, E_{max}: maximal contracting effect (a negative sign means relaxation), p < 0.05, **p < 0.01, ***p < 0.001.

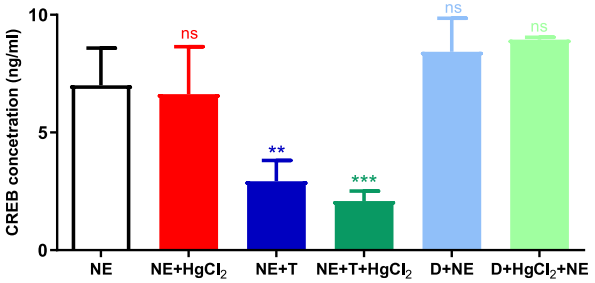

Fig. 5. Changes in the concentration of cAMP response element binding protein (CREB) after $HgCl_2$, terbutaline (T) and doxazosin (D) treatment in norepinephrine (NE)-evoked contraction samples. ns p > 0.05, **p < 0.01, ***p < 0.001 compared to the NE-treated uterus samples.

terbutaline, and doxazosin, *in vitro*. The $HgCl_2$ treatment did not induce changes in the CREB level in the NE-treated uterus. β_2-receptor agonist, terbutaline, significantly decreased the CREB protein level after NE treatment, which was further enhanced by $HgCl_2$. In contrast, α_1-antagonist doxazosin did not cause significant changes in CREB protein levels with or without $HgCl_2$ (Fig. 5).

4. Discussion

In our work, we investigated the AQP5 channel as a factor involved in uterine contraction in the pregnant uterus, hypothesizing that it could represent a new drug target for tocolysis. Various signaling pathways are recognized to regulate the expression of aquaporins (AQPs) in animal species. Two possible pathways – receptor and osmotic – are hypothesized in the pregnant uterus. Our research group has previously demonstrated an osmotic pathway influencing uterine contractions in the late-pregnant rat uterus via TRPV4 [14,15]. In our present studies, we focused on the receptor pathway, a putative cooperative pathway with the adrenergic system.

The direct pharmacological examination of the AQP5 channel is difficult because there are no subtype-selective drugs available to perform *in vivo* experiments and investigate the physiological role of AQP5. Therefore, AQP5 siRNA treatment provided a good opportunity to directly study the role of the AQP5 channel in the onset of labour. siRNA (small interfering RNA) can regulate gene expression through the phenomenon of RNA interference. RNA interference is a biological mechanism through which double-stranded RNA facilitates gene silencing by specifically targeting and degrading complementary messenger RNA (mRNA) [16]. The decrease in AQP5 mRNA and protein expression following AQP5 siRNA treatment indicates that the siRNA treatment was effective and gene silencing occurred. The increase in *in vitro* contractions and the change in the time of birth, with AQP5 siRNA-treated animals giving birth significantly earlier, support our hypotheses [10] that decreased AQP5 expression led to increased uterine contractions, resulting in an earlier onset of parturition.

In the kidney, the translocation of AQP2 to the apical plasma membrane by vasopressin is known to be mediated by cAMP via the

V2 receptor [17]. On the basis of this, several studies have already shown that cAMP affects the expression of certain aquaporins, including AQP5. In porcine myometrial tissues, AQP5 mRNA expression decreased after both short (3h) and long (24h) incubation with cAMP in the mid-luteal phase and increased during luteolysis after short incubation [18]. In an *in vitro* system of mouse lung epithelial cells (MLE-12), a significant increase in AQP5 mRNA and protein levels was observed as a result of cpt-cAMP treatment [19]. Elevated levels of cAMP in bronchial epithelia stimulate PKA to phosphorylate AQP5, which causes these water channels to translocate to the cell membrane [12,20]. Terbutaline is a β2-adrenergic receptor agonist tocolytic agent used in human therapy, which exerts its effect via cAMP mediation. The action of the β2 receptor commences upon the activation of the receptor by an agonist, which results in the dissociation of the alpha subunit of the Gs protein. This subunit subsequently reattaches to adenylate cyclase, thereby stimulating the enzyme to catalyze the conversion of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP), which functions as a second messenger within the cell. In turn, cAMP activates two pathways that facilitate smooth muscle relaxation. The first pathway involves the release of the catalytic subunit of the protein kinase A enzyme, which phosphorylates various enzymes that promote the relaxation of smooth muscle. The second pathway entails a reduction in intracellular calcium concentration, achieved by inhibiting the influx of calcium from the extracellular environment, preventing the efflux of calcium from intracellular stores, and promoting the sequestration of calcium ions within the cytoplasm, thereby inhibiting muscle contraction [21]. We demonstrated time-dependent alterations in the expression of AQP5 mRNA and protein following terbutaline treatment. Presumably, the increase in cAMP levels resulting from terbutaline treatment increased AQP5 expression in the late-pregnant uterus, similarly to other tissues, e.g. lung cells. Doxazosin is also a drug acting on the adrenergic system, an α1-selective antagonist used to treat hypertension and benign prostatic hyperplasia [22,23]. Based on pharmacological logic and the experimental results, doxazosin has both smooth muscle relaxant and tocolytic effects [24]. The activation of the α1-adrenoreceptor, and through it IP3 and ryanodine receptors, leads to the translocation of the AQP5 channel from the intracellular space to the apical membrane, together with lipid rafts in the rat parotid gland [25]. α1-adrenoreceptor blocker doxazosin has no significant effect on AQP5 expression, presumably because it does not influence the level of cAMP in pregnant rat uterus.

AQP5 is a mercury-sensitive aquaporin channel [26]. Certain aquaporins possess cysteine residues within the E loop adjacent to the pores, which confer functional sensitivity to mercury. The interaction of mercury with this cysteine leads to the collapse of the pore, thereby inhibiting water movement through the channel [27]. It is commonly established that all types of mercury have harmful effects on mammals [28]. Therefore, it is not appropriate for *in vivo* experiments, but the *in vitro* investigation of the results of mercury treatment can provide important scientific information. Based on these, we investigated the impact of $HgCl_2$ on the pregnant rat uterus in an isolated *in vitro* organ bath system. Contractions in spontaneous 22-day-old uteri were increased by $HgCl_2$ treatment. This result confirmed our earlier results [10] that inhibition of the AQP5 channel activation or decrease in the expression – with $HgCl_2$ in this case – led to an increase in uterine contractions. The effect of mercuric chloride on the contractions of the pregnant uterus was investigated not only in the case of spontaneous contractions but also in the presence of norepinephrine. Norepinephrine (NE) is a non-selective adrenergic receptor (AR) agonist with a significant agonist effect on alpha1 and 2 adrenergic receptor subtypes compared to beta (1)-receptors. α-ARs mediate smooth muscle contraction in the uterus, as can be seen in our *in vitro* studies. NE-induced contractions (AUC) significantly decreased after $HgCl_2$ treatment, probably because of the insignificant β-AR effect, which could not be affected by mercuric chloride via AQP5. To map the molecular biology background of these changes, we examined the changes in AQP5 mRNA and protein expression. Further evidence was obtained here that high uterus contraction correlates with low AQP5 mRNA and protein expression.

A dose-dependent inhibitory effect of the β_2-AR agonist terbutaline on uterine contraction in an isolated organ bath system in the presence of NE was determined, which inhibitory effect was potentiated by the AQP5 inhibitor $HgCl_2$.

The dose-dependent selective role of adrenergic receptors was investigated with NE alone or in the presence of the β-AR antagonist propranolol, which did not affect the NE α_1-AR-mediated uterine contraction effect. Mercuric chloride (extremly, in the presence of propranolol) decreased pregnant uterus contactions, presumably through changes in AQP5 function. The uterus relexant effect of terbutaline was enhanced by $HgCl_2$, especially at higher doses of NE. Alpha1-AR receptor antagonist doxazosin mediated the uterus relexation effect on free α-ARs, and when $HgCl_2$ was added to this system, it further enhanced the uterine relaxant effect through the AQP5 channel, probably due to the non-selective adrenergic receptor inhibitory effect of NE. These *in vitro* results suggest that the presence of $HgCl_2$ affects AQP5 expression and, through this, the development of uterine contractions.

The transcription factor CREB (cAMP response element binding protein) regulates cellular responses, including differentiation, survival, and proliferation [29]. Adenylate cyclase (AC), which is activated by the stimulation of cellular G-protein-coupled receptors through neurotransmitters, elevates the levels of cyclic adenosine monophosphate (cAMP). This increase in cAMP subsequently activates protein kinase A (PKA). The catalytic subunits of PKA then translocate to the nucleus, where they phosphorylate cAMP response element-binding protein (CREB) at serine 133 [30,31]. The activation of β2-ARs increases intracellular levels of cAMP, and CREB is phosphorylated in response to this second messenger [32], these confirm our *in vitro* data showing the uterus relaxant effect of terbutaline with a decrease in CREB levels. The presence of $HgCl_2$ significantly induced the decrease of CREB by terbutaline, presumably that this change in cAMP levels may affect AQP5 function.

Alpha1-AR selective NE has a weak effect on CREB phosphorylation, as it does not influence the cAMP level but increases the concetration of inositol triphosphate and diacylglycerol. As expected, doxazosin treatment did not significantly change the level of CREB, similarly to NE.

5. Conclusion

Due to the wide range of causes of spontaneous preterm birth, identification and prevention are difficult and, in some cases, not

possible. The solution to this problem would be a better understanding of the mechanism of preterm birth. We proved the role of AQP5 in the induction of birth by the AQP5 knockdown animal model and the cooperativity between AQP5 channels and the adrenergic system through cAMP. However, the exact mechanism of the synergic effect needs further investigation. In our opinion, the combination of an AQP5 channel inhibitor and a β-adrenergic receptor agonist (e.g., terbutaline) can be considered a good therapeutic combination for evolving a new tocolytic protocol.

Data availability

All data accessed and analyzed in this study are available in the article and its supplementary materials. Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Dr Eszter Ducza (ducza.eszter@szte.hu).

CRediT authorship contribution statement

Kata Kira Kemény: Writing – original draft, Project administration, Methodology, Investigation. **Adrienn Seres-Bokor:** Methodology, Investigation. **Tamara Barna:** Methodology. **Mohsen Mirdamadi:** Methodology. **Róbert Gáspár:** Writing – original draft, Methodology, Conceptualization. **Andrea Surányi:** Methodology, Conceptualization. **Eszter Ducza:** Writing – original draft, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37329.

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