# Examination of the contractility of human placental blood vessels in vitro

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# 1. Introduction

It is very important to maintain the placental circulation at the best level possible, though the mechanisms responsible for the regulation of placental blood flow are poorly understood. A detailed knowledge is needed of the direct effects on the human placental vasculature of all the drugs given during pregnancy and during delivery. There are several reports on the direct placental vascular effects of different chemical substances: endothelin-1, prostaglandin F2-alpha, sodium nitroprusside, histamine, ouabain, serotonin and ketanserin.
Electric field stimulation (EFS) is widely used in physiological and pharmacological research. Several reports have already been published on investigations of the physiological and pharmacological behavior of different vessels by EFS, but we have found no data on the application of EFS to study human placental blood vessels in the literature.
Cloning and pharmacological data have revealed that the alpha1-ARs can be classified into the three subtypes: alpha1A-, alpha1B-, and alpha1D-ARs. Each of the alpha1-AR subtypes has been found to mediate distinct physiological function. The adrenergic system plays an important role in the regulation of the uterine motor activity. Contraction is mediated by the alpha-ARs in the uterine smooth muscle. This provides a theoretical possibility for the use of alpha1-AR blockers as tocolytic agents, which has been verified in animal studies. Beta-ARs are also involved in uterine relaxation, which is reflected in clinical obstetrics practice by the frequent application of beta2-agonists as tocolytics. The use of beta2-agonists and the potential use of alpha1-AR blockers as tocolytics raise the question how they influence placental circulation. We have found only limited data on the density, diversity and distribution of the alpha1- and beta2-ARs in the human placental vasculature in the literature.
However, all the aforementioned objectives of ours consideres the investigation of only vasodilatator agents, yet there are agents, which can be possibly dangerous during pregnancy and delivery by possessing the opposite (vasoconstrictor) characteristics on human placental blood vessels. The main side-effect of the chronic application of recombinant human erythropoietin (rHuEPO) as therapy for hemodialyzed patients has been reported to be the development or aggravation of hypertension. The development of hypertension directly after application suggested that direct pressor mechanisms may be involved. The direct vasoconstrictor effect of erythropoietin (EPO) has been reported by various authors in numerous animal studies, but we have found no data on the direct effect of EPO on human placental blood vessels in the literature. The EPO receptor (EPO-R) protein and its mRNA, classically found in erythroid precursor cells, have been described in other cell types, including endothelial cells of the fetoplacental vasculature. Considerable interest has also focused on the potential for angiotensin convertase enzyme (ACE) inhibitors and angiotensin II (ANG II) receptor antagonists to affect the response to EPO.

# 2. Objectives

1. To develop an EFS model suitable for investigations of the contractility of placental veins and arteries, and of the direct effects of different pharmacological agents on the human placental blood vessels. Comparison of the physiological and pharmacological behavior of placental vessels to non-placental blood vessels (e.g. rat vessels) was also aimed.
2. To test the pharmacological capability of the model.
3. To determine the expression and pharmacological reactivity of the alpha1- and beta2-ARs in the human term placental vasculature.

4. To examine the direct effects of rHuEPO on isolated human placental blood vessels. To investigate the effects of captopril and losartan on the rHuEPO-induced contractions of isolated human placental veins and arteries.

# 3. Materials and Methods

### 3.1. Sampling and Preparation

Placentas were obtained from the Delivery Room of the Department of Obstetrics and Gynecology, University of Szeged. The presence of any pathological conditions prevented the inclusion of that very placenta in the study. They were transferred in 500 ml icy Krebs-Henseleit buffer, and the experiments were begun 10-30 min after birth. The placentas were selected randomly for study inclusion from term, singleton pregnancies of healthy Caucasian mothers that ended with uncomplicated deliveries. After the umbilical cord had been cut off, thin polyethylene cannulae were led into the vein (with larger diameter) and the two arteries (with smaller diameters) in the stub, in order to separate the veins and arteries on the fetal surface of the placenta. The vessels were prepared for in vitro measurement according to the method outlined by Angus and Wright. Rings 1-1.2 mm in diameter were dissected from the identified veins and arteries just before their heading towards the stem villi.
Comparative experiments on rat mesenterial arteries were also undertaken. The rat mesenterial arteries were dissected from 200-220 g female Sprague-Dawley rats through laparotomy.

### 3.2. EFS Studies

The rings were mounted between two platinum electrodes in an organ bath. The passive force was set at approximately 3.75 g and 3.25 g for veins and arteries, respectively. The optimal degree of stretch was ascertained by determining a contraction versus passive force curve in response to an EFS stimulus with a stimulating potential of 30 V, a period time of 4 s and a pulse width of 80 ms. Using the same technique the optimal passive force for rat mesenterial arteries turned out to be approximately 2.2 g.
Contractions were elicited by a digital, programmable stimulator. The force of the vessel rings was measured with a gauge transducer, and recorded by an ISOSYS Data Acquisition System. The optimal period time was determined by decreasing the applied period time until the individual contractions fused to produce a smooth contractile response. 20 and 30 V were used as stimulating potentials. The pulse width values to be used to elicit half-maximal contractions were determined by applying stimuli of different durations (pulse width: 25, 50, 100 and 200 ms).
The role of calcium and calcium channels was assessed by conducting experiments in $Ca^{2+}$-free medium and with blockers of both different ion channels and the intracellular $Ca^{2+}$-stores. To test the pharmacological capability of the model, the effects of $NaNO_2$ was examined. To test the pharmacological reactivity of the different AR-types terbutalin, fenoterol, ritodrine, WB4101, BMY7378 and urapidil were studied.

### 3.3. Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) Studies

#### 3.3.1. Tissue isolation

More placental vessel tissues for RT-PCR studies were rapidly dissected in ice-cold saline containing recombinant ribonuclease inhibitor.

#### 3.3.2. Total RNA preparation

Total cellular RNA was isolated by extraction with guanidinium thiocyanate-acid-phenol-chloroform. After precipitation with isopropanol, the RNA was treated with RNase–free DNase I for 30 min at 37 °C, re-extracted with phenol, precipitated with ethanol, washed with 75% ethanol and then resuspended in diethylpyrocarbonate-treated water, and the RNA concentration was determined by optical density measurements at 260 nm.

### 3.3.3. RT-PCR

The RNA was denatured in a reaction mixture containing RNase inhibitor, dNTP, oligo(dT), Tris-HCl, KCl and $MgCl_2$. After the mixture had been cooled, M-MLV Reverse transcriptase, RNase H Minus was added, and the mixture was incubated at 37 °C for 60 min and then at 72 °C for 10 min.

PCR was carried out with cDNA, ReadyMix REDTaq PCR reaction mix and sense and antisense primers. A human GAPDH probe was used as an internal control in all samples. The PCR was performed with a PCR Sprint thermal cycler. The PCR products were visualized by performing the electrophoresis on gel containing ethidium bromide. Quantitative analysis was performed by densitometric scanning of the gel with the KODAK EDAS290 system. An AR/GAPDH amplification ratio was calculated for each RNA pool.

# 4. Results

### 4.1. EFS Studies

EFS induced contractions in the human placental blood vessel rings. The optimal period time was found to be 5 s.

On stimulation at 20 V, the pulse width location at half maximal force was above 500 ms for both veins and arteries. These values were so extremely high that the curve was considered unsaturable. The contractions of the veins were significantly stronger than those of the arteries at all applied pulse widths ($p<0.05$).

On stimulation at 30 V, the optimal pulse width (the pulse width location at half maximum force) for veins and arteries was 100 ms and 119 ms, respectively. Bubbling through physiological in utero hypoxic gases significantly enhanced the contractile responses of both vein and artery rings to EFS at all applied pulse widths, though the optimal pulse widths were not changed significantly: 99 ms and 105 ms for veins and arteries, respectively.

After reaching half-maximal contraction, the placental vessel rings exhibited a spontaneous relaxation. The arteries displayed a slightly greater relaxation, but the difference between the relaxation of the veins and the arteries was not significant ($p>0.05$). The proportion of the spontaneous relaxation as a function of time after the half-maximal contraction was reached could be described by third-degree polynomial functions. Bubbling through physiological in utero hypoxic gases has not altered the spontaneous relaxation of the veins, but those of the arteries has been reduced to zero.

EFS induced contractions also on the rat mesenterial arterial rings. After reaching half-maximal contraction, the rat mesenterial rings also exhibited a spontaneous relaxation. The proportion of the spontaneous relaxation as a function of time could be described by another third-degree polynomial function. Controversially to the placental vessel rings, in utero gases significantly blunted the forces of the contractions at all applied pulse widths, but it did not alter the spontaneous relaxation of the rat mesenterial arterial rings.

To test the pharmacological capability of the model, the effects of $NaNO_2$ was examined. It antagonized the contractions of the placental vessel rings in a dose-dependent manner, but the efficacy of $NaNO_2$ was significantly decreased by the in utero gases.

The contractions were not changed by tetrodotoxine. Pretreatment with verapamil or $Ni^{2+}$ inhibited the contractions to a magnitude of 63.81 ± 7.69 % and 88.36 ± 12.17 %, respectively. Combined verapamil and $Ni^{2+}$ treatment inhibited the contractions to a similar magnitude as $Ni^{2+}$ treatment in itself. In $Ca^{2+}$-free medium after combined cyclopiazonic acid and $Ni^{2+}$ treatment it was not possible to elicit contractions with EFS.
Beta2-mimetics antagonized the EFS induced contractions of the human placental arterial rings in a dose dependent manner. The relaxant effects of the beta2 mimetics were significantly less marked in case of the veins at all applied doses.
WB4101 and BMY7378 both antagonized the EFS induced contractions of the human placental arterial rings in a dose dependent manner. The relaxant effects of WB4101 and BMY7378 were significantly less marked in case of the veins at all applied doses. Urapidil antagonized the EFS induced contractions of both the human placental arterial and vein rings in a dose-dependent manner. Inhibition was lower in case of the veins at all applied doses, but none of the differences were significant.

### 4.2. RT-PCR Studies

The expression of beta2-AR mRNA was significantly higher in the arteries compared to the veins.
The expression of alpha1A- and alpha1B- AR mRNA were significantly higher in the arteries compared to the veins. Significant difference in the amount of alpha1D-AR mRNA between veins and arteries was not possible to find. The expression of ARs were predominant in the human placental arteries. The preponderance of alpha1A- and alpha1B-AR mRNA compared to the alpha1D-AR mRNA was proved both in the arteries and in the veins.

### 4.3. EPO Studies

rHuEPO evoked contractions in human placental blood vessel rings. At all applied doses the effect of rHuEPO on the placental veins was significantly more marked, as compared to that on the arteries.
Incubation of the vessel rings with captopril did not affect their contractile response to rHuEPO. In contrast, losartan significantly blunted the contractile responses of the vessel rings.

## 5. Conclusions (Summary)

**1.** EFS has been successfully applied for the study of human placental vessels, which is therefore a new experimental possibility for investigations of the direct placental vascular effects of different pharmacological agents. The optimal parameters of our EFS model for human placental blood vessels are as follows: gases simulating physiological intrauterine hypoxia bubbled through Krebs-Henseleit buffer; passive forces are 3.25 g and 3.75 g for arteries and veins, respectively; stimulating potential: 30 V, period time: 5 s; pulse widths are 105 ms and 99 ms for arteries and veins, respectively.
**2.** Controversially to other; non-placental vessels in utero physiological hypoxic circumstances has a stimulatory/enhancing effect on the contractility of human placental vessels.
**3.** The direct, non-neurogenic contractile effect of EFS on isolated human placental blood vessel rings mainly depends on the influx of extracellular $Ca^{2+}$ via voltage operated $Ca^{2+}$-channels, partly on the mobilization of intracellular $Ca^{2+}$-stores, and on a mechanism independent of intracellular $Ca^{2+}$-concentration elevation. This mechanism independent of intracellular $Ca^{2+}$-concentration elevation is likely to be the influx of other extracellular cations besides $Ca^{2+}$.

**4.** Beta2-, alpha1A-, alpha1B- and alpha1D-ARs are all involved in the regulation of human term placental vessels' contractility. This involvement seems significantly more marked in the case of the arteries compared to the veins. There is relationship between the mRNA expression and the pharmacological reactivity for ARs. The alpha1A- and the beta2-ARs seem to play the major role in the regulation of both the human term placental arteries and veins as regards the AR-subtypes, nevertheless alpha1B-ARs also seem to play a considerable role in this respect. The role of the alpha1D-ARs seem to be moderate compared to the alpha1A-, alpha1B- and beta2-ARs in the regulation of the contractility of both the human term placental arteries and veins.
**5.** On the basis of our in vitro studies the use of beta2-mimetics and the potential use of alpha1-blockers as tocolytics can be considered secure or even beneficial concerning placental blood flow.
**6.** rHuEPO has a direct and dose-dependent contractile effect on human placental blood vessels. EPO might participate in one of the humoral mechanisms involved in the control of the placental vascular bed. $AT_1$ receptors are needed to mediate the contractile response of human placental blood vessels to rHuEPO.
**7.** EPO might also be involved, in part, in the pathogenesis of preeclampsia and intrauterine growth restriction.

# 6. Publications Connected to the Thesis


1. Resch Béla Endre, Gáspár Róbert, Falkay György: A placentaerek kontraktilitásának vizsgálata elektromos téringerléssel. (2002) *Magyar Nõorvosok Lapja* 65, 161-167.
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   JIF: 2.196
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   JIF: 2.871
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   JIF: 2.296


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