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Pregnancy-induced hypertension is associated with down-regulation of Kir6.1 in human myometrium

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ABSTRACT

It is generally accepted that activity of K^+ channels maintain resting membrane potential and uterine quiescence during pregnancy, which is, at least in part, mediated by down-regulation of ATP-sensitive K^+ (K_{ATP}) channels. Pregnancy-induced hypertension (PIH) is associated with pre-term and late pre-term labour. Here, we have used real time RT-PCR to compare mRNA levels of K_{ATP} channel subunits in PIH parturient and control parturient. We have found that Kir6.1, a pore forming, myometrial K_{ATP} channel subunit is down-regulated in PIH patients. This could perfectly explain increased rate of pre-term labour in patients suffering from PIH.

1. Introduction

ATP-sensitive K^+ (K_{ATP}) channels couple intracellular metabolism with the membrane excitability and they perform crucial physiological roles such as cardioprotection, insulin secretion and regulation of muscle tone [1,2]. Structurally, K_{ATP} channels are composed of pore-forming inward rectifier, Kir6.1 or Kir6.2, and a regulatory, ATP-binding subunit, SUR1, SUR2A or SUR2B. The properties of these channels are different in various tissues due to the combinations of the subunits forming the channel [1,2]. Human myometrium express Kir6.1 and Kir6.2 as a pore-forming subunits and SUR1, SUR2A and SUR2B as regulatory subunits [3]. Myometrium is normally in a quiescent state during pregnancy, but dramatically increase its contractile activity during labour. The process responsible for uterine quiescence during pregnancy and increased contractile activity for labour are complex and influenced by many factors. It is generally accepted that activity of K^+ channels maintain resting membrane potential and uterine quiescence during pregnancy [4]. In myometrium, transcripts of all possible subunits of ATP-sensitive K^+ (K_{ATP}) channels, Kir6.1, Kir6.2, SUR1 and SUR2B, were identified [3]. It has been shown that K_{ATP} channels are down-regulated in myometrium in late pregnancy, which is associated with increase in uterine excitability and consequent induction of labour contractions in term pregnancy [3,5]. Pregnancy-induced hypertension (PIH) is associated with pre-term and late pre-term labour [6,7].

Taking into the consideration presence and significance of K_{ATP} channels in myometrium [3–5] and PIH association with pre-term labour [6,7], we hypothesised that expression of these channels may be affected by hypertension in pregnancy.

2. Methods

2.1. Patients and samples

Patients were recruited in the Department of Obstetrics and Gynaecology, Medical School, University of Belgrade. The study was approved by the Research Ethics Committee, Clinical Centre (No. 1206/41), and recruitment was carried out by provision of information sheets and by obtaining written informed consent. Pregnancy-induced hypertension (PIH) was defined as the development of new hypertension (blood pressure equal or above 140/90 mm Hg) in a pregnant woman following 20 weeks' gestation without the presence of protein in the urine or other signs of pre-eclampsia. Biopsies of myometrium of 10 pregnant women diagnosed with PIH (age: 29.80 ± 1.70, n = 10) and age-matched (age: 29.40 ± 1.99, n = 10; P = 0.880) pregnant control women were excised from the midline of the upper lip of the uterine incision made at Caesarean section. All women were of the Caucasian race. Average gestational age was 40.1 ± 0.01 weeks (n = 10) for normotensive parturient and 39.2 ± 1.9 weeks (n = 10) for parturient diagnosed with PIH (P = 0.372). BMI and pregnancy parity statistically was non-significantly different between the two groups (BMI: Control:

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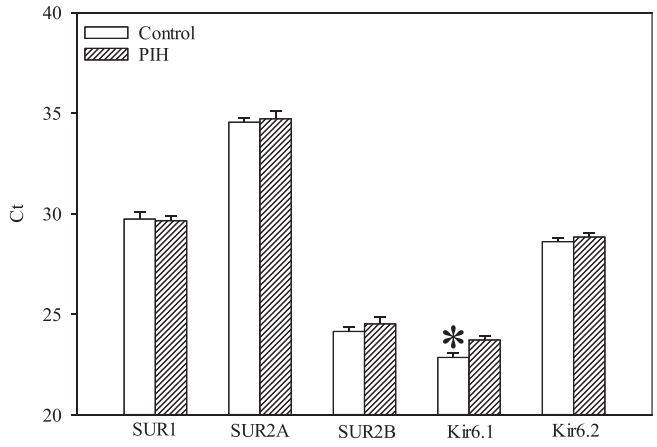

Fig. 1. mRNA of K_{ATP} channels subunits in control and PIH parturient. A graph depicting real time RT-PCR cycling thresholds (Ct) for different K_{ATP} channel subunits in myometrium. Each bar represents mean ± SEM (n = 10 for each).*P < 0.01.

26.3 ± 1.6 (n = 8); PIH: 32.6 ± 1.6 (n = 6), P = 0.075; Pregnancy parity: Control: 1.5 ± 0.3 (n = 8); PIH: 2.0 ± 0.2 (n = 6), P = 0.694). No evidence of inflammation/infection was observed in any patients apart from one case of genital herpes and one case of vaginal/cervical infection (both PIH patients). All previous pregnancies resulted in life births. Caesarean sections were elective pre-labour performed due to fetopelvic disproportion and previous Caesarean section. Upon removal, tissue samples were rinsed with physiological solution and immediately frozen in liquid nitrogen at −80 °C. Women suffering from multiple diseases were excluded from the study.

2.2. RNA preparation and real time RT-PCR

Total RNA was extracted from myometrial tissue using TRIZOL reagent (Invitrogen, Paisley, UK) according to the manufacturer's instructions. Extracted RNA was further purified with RNeasy Mini Kit (Qiagen, Crawley, UK). Real time RT-PCR was performed as described previously in details [5].

2.3. Statistical analysis

Threshold cycles (Ct) obtained by real time RT-PCR are presented as mean ± S.E.M. Mean values were compared using *t*-test or Mann-Whitney Ranking Test where appropriate using SigmaPlot 12.5 (Jandel Scientific, USA). P < 0.05 was considered statistically significant.

3. Results

No statistically significant difference in real time RT-PCR cycling threshold (Ct) values was observed when compared SUR1, SUR2A, SUR2B and Kir6.2 in myometrium from normotensive parturient and parturient diagnosed with PIH (Ct values for Control vs PIH: SUR1: 29.74 ± 0.33 vs 29.66 ± 0.22, P = 0.843; SUR2A: 34.55 ± 0.22 vs 34.72 ± 0.37, P = 0.970; SUR2B: 24.15 ± 0.20 vs 24.53 ± 0.33, P = 0.342; Kir6.2: 28.62 ± 0.29 vs 28.85 ± 0.21, P = 0.540; n = 10 for each; Fig. 1). On the other hand, Kir6.1 subunit was significantly down-regulated. Ct values were 22.86 ± 0.21 vs 23.73 ± 0.18, P = 0.003; n = 10 for each; Fig. 1).

4. Discussion

PIH is a major cause of maternal and fetal morbidity and mortality [8] and association between PIH and pre-term birth has been reported many times [6–8]. It is well established that SUR1, SUR2A; SUR2B, Kir6.1, Kir6.2 mRNAs are present in human myometrium [3–5], which is in agreement with the present study. The presence of mRNAs for all five subunits has been reported in other musces, including skeletal and heart muscle [9,10]. Regardless of the presence of all five subunits, in each of these tissues, it has been suggested that only two types of subunits physically associate to form functional K_{ATP} channels. In human myometrium, it has been shown that SUR2B/Kir6.1 is the combination that make functional K_{ATP} channels [3,5]. These channels maintain resting membrane potential and uterine quiescence [4]. In late pregnancy, K_{ATP} channels are down-regulated in myometrium, which increase uterine excitability and contribute to induction of labour contractions in term pregnancy [3,5]. Here, we have found that in patients suffering of PIH there is further down-regulation of Kir6.1. Kir6.1 is a pore-forming myometrial K_{ATP} channel subunit and decreased number of these channels increases uterine excitability leading to increased risk of labour induction. In this respect, down-regulation of Kir6.1 perfectly explains the mechanism underlying pre-term labour in PIH. In the cohort of partiurent that did not have PIH, we had only one pre-mature labour and in this particular partiurent the threshold cycle for Kir6.1 was 23.50, which was more similar to average value for PIH (23.73) than non-PIH (22.86) partiurent. This is in agreement with the notion that levels of K_{ATP} channels in the myometrium is important factor regulating timing of labour. Kir6.1 forms K_{ATP} channels not only in myometrium, but also in vascular smooth muscle [2]. Activation of vascular K_{ATP} channels induces hypotension while their decreased numbers are associated with hypertension [2]. It is quite possible that down-regulation of Kir6.1 causes both hypertension and interferes with myometrium contractility during pregnancy.

A limitation of our study is that we did not directly measure protein levels of K_{ATP} channel subunits. However, it should be pointed out that in previous studies mRNA levels of channel subunits always corresponded to their protein levels.

In conclusion, this is the first report to demonstrate a link between K^+ channel levels in myometrium and PIH, which, in turn, could explain increased rate of pre-term labour in patients suffering from PIH.

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Declaration of Competing Interest

The authors have no conflicts of interest to disclose in relation to this paper.

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