Pharmacological evaluation of bradykinin effect on human umbilical artery in normal, hypertensive and diabetic pregnancy

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Abstract: The objective of this investigation was to compare bradykinin (BK) action on isolated intact or denuded human umbilical artery (HUA) in normal pregnancy, pregnancy-induced hypertension (PIH) and gestational diabetes mellitus (GDM). Bradykinin contracted HUA in a concentration-dependent manner in all investigated groups. Control BK contractions were unchanged by L-NOARG (NO-synthase inhibitor), glibenclamide (K\textsubscript{i} channel blocker), or des-Arg\textsuperscript{9} (leu\textsuperscript{9})-BK (B\textsubscript{i} antagonist), while were reduced by indomethacin (cyclooxygenase inhibitor) or nifedipine (Ca\textsuperscript{2+} channel blocker). After endothelial denudation in GDM, concentration-response curve for BK was shifted to the left in relation to control HUA from normal pregnancy. OKY-046 (thromboxane A\textsubscript{2}-synthase inhibitor) displaced concentration-response curve for BK to the right in PIH, whereas reduction in maximal contraction was obtained in HUA from GDM. Ouabain (Na\textsuperscript{+}/K\textsuperscript{+}-ATPase inhibitor) contracted HUA prior to BK addition in all groups. Apamin (small conductance K\textsubscript{Ca} channel blocker), TEA (non-selective K\textsuperscript{+} channel blocker) or Ba\textsuperscript{2+} (K\textsubscript{Ca} channel blocker) augmented maximal BK contractions in normal pregnancy, PIH and GDM, respectively. HOE 140 (B\textsubscript{i} antagonist) produced concentration-dependent inhibition of BK effect in all groups. Collectively, in HUA from all groups BK evoked vasoconstriction via smooth muscle B\textsubscript{i} receptors. Intact endothelium provided additional modulation of BK contraction in GDM. Contribution of contractile cyclooxygenase products to BK action was demonstrated, and in PIH and GDM thromboxane A\textsubscript{2} was also involved. Voltage-gated Ca\textsuperscript{2+} channels and Na\textsuperscript{+}/K\textsuperscript{+}-ATPase contribute to the BK contraction, and to the regulation of basal vascular tone, respectively. Diverse K\textsuperscript{+} channels modulate BK contraction in HUA by preventing excessive vasoconstriction.

Key words: bradykinin, human umbilical artery, pregnancy-induced hypertension, gestational diabetes mellitus

Introduction

Blood flow in fetoplacental circulation is significantly influenced by vasoactive substances, some of which are locally synthesized or are delivered via systemic circulation. It has been established that several vasoactive agents, such as prostanoids or serotonin [7, 20, 25], may change smooth muscle tone of human umbilical blood vessels, with or without notable participation of endothelium-derived relaxing and contractile factors.
Bradykinin (BK) is one of many vasoactive substances contributing to the physiological preservation of cardiovascular system functioning. Thus, this kinin is known to contribute to labor progression by inducing constriction of umbilical blood vessels and ductus arteriosus, whereas reducing the vascular resistance in pulmonary circulation [2, 3, 11]. However, an augmentation of kininase I activity and resulting degradation of BK correlates with the lower concentration of this kinin in fetoplacental circulation of women with pathological conditions characterized by chronic hypoxia, such as preeclampsia [34]. The function of endothelial and smooth muscle cells is significantly impaired in umbilical vessels from preeclamptic pregnancies, too [9, 23]. In addition, endothelial dysfunction is evident in women with normal or increased body weight after prior pregnancy with gestational diabetes mellitus [5, 18]. Thus, it may be anticipated that the responsiveness of umbilical blood vessels to BK might be altered in pathological conditions associated with pregnancy.

Since the umbilical artery plays an essential role in adequate perfusion and nutrition of the placenta, and taking into account that BK-induced effects in this blood vessel are still not fully understood, the present experiments were undertaken in order: (1) to examine and to compare BK action on isolated human umbilical artery (HUA) obtained at labor from women with normal pregnancy, pregnancy-induced hypertension (PIH) and gestational diabetes mellitus (GDM); (2) to investigate the contribution of endothelial vascular layer and potential involvement of endothelial factors in BK-evoked vascular response; (3) to establish if potassium channels are important for BK’s transduction mechanism and (4) to determine the population of kinin receptors involved in the BK-induced effect.

Materials and Methods

Patients

Preparation of human umbilical artery

The experiments were performed on human umbilical arteries isolated from the middle part of the umbilical cords that were obtained immediately after the vaginal delivery in women with normal pregnancy or after Cesarean section in women with pathological pregnancy. Our investigation enrolled 44 women with normal, full-term pregnancy (middle maternal age = 27, middle gestational age at the delivery = 39.1 weeks), as well as 13 women with pregnancy-induced hypertension (middle maternal age = 29, middle gestational age at the delivery = 38.5 weeks) and 11 women with gestational diabetes mellitus (middle maternal age = 31, middle gestational age at the delivery = 38.8 weeks). Pregnancy-induced hypertension (the primary hypertensive disorder in pregnancy – gestational hypertension) has been confirmed by elevated blood pressure detected for the first time after midpregnancy (on at least two occasions), and not associated with proteinuria [17]. On the other hand, the presence of glucose intolerance has been initially tested in pregnant woman without known diabetes by glucose challenge test, and gestational diabetes mellitus was additionally confirmed by a standard oral glucose tolerance test [4]. All women were hospitalized and followed-up at the Institute of Obstetrics and Gynecology, Medical Faculty – Belgrade. The present study has been approved by the Ethics Committee at the Medical Faculty in Belgrade.

Only the remnant tissues, which would have been otherwise disposed of, have been utilized. The obtained umbilical cords were kept overnight at 4°C and the experiments on umbilical artery have been carried out within 24 h after the delivery [5, 38].

At the beginning of each experiment, umbilical artery has been isolated from the Wharton’s jelly, dissected out of the surrounding tissue and cut into 4 mm long ring segments. During this procedure special care was taken not to stretch the blood vessel, and from each artery up to 12 vascular rings have been prepared. These have been intended to be randomly allocated for the experiments with different pharmacological blockers or receptor antagonists. The endothelium was removed from some rings by gentle rubbing the intimal surface with stainless-steel wire and the absence of endothelium was confirmed histologically [7]. Ring preparations were mounted between two stainless-steel triangles in an organ bath containing 20 ml Krebs’ bicarbonate solution (37°C, pH 7.4), aerated with 95% O₂ and 5% CO₂. One of the triangles was attached to a displacement unit allowing for a fine adjustment of tension, and further connected to a force-displacement transducer (Hugo Sachs Elektronik F30 Type 372, Freiburg, Germany). Isometric tension was continuously recorded on a Rikadenki R-62 multi-pen electronic recorder (Rikadenki Kogyo, Japan).
Co., Ltd, Tokyo, Japan). Vascular rings were allowed to equilibrate for 90 min in Krebs’ bicarbonate solution. The organ baths were washed with fresh buffer solution every 15 min. After 90 min, each ring was gradually stretched during the next 30 min to the resting tension of 2 g ≈ 20 mN [1, 38], and additionally equilibrated for 30 min before experimentation.

Drugs and solutions

The Krebs’ bicarbonate solution had the following composition (in mM): NaCl 115.0; KCl 4.6; CaCl₂ 2.5; MgSO₄ 2.5; KH₂PO₄ 1.2; NaHCO₃ 25.0; glucose 11.1. The following drugs were used: bradykinin, apamin, BaCl₂, nifedipine, des-Arg⁹(lei⁸)-BK (ICN, Irvine, CA, USA), indomethacin, glibenclamide, ouabain, D-Arg[γHyp³,Thi⁵,D-Tic⁷,Oic⁸]bradykinin (HOE 140) (Sigma, St Louis, USA), N⁵-nitro-L-arginine (L-NOARG) (RBI, Natick, MA, USA), tetraethylammonium bromide (TEA) (Serva, Heidelberg, Germany) and (E)-3-(4-(1-imidazolylmethyl)phenyl)-2-propenoic acid) – OKY-046 (Ono Pharmaceutical, Osaka, Japan). All agents were dissolved in distilled water (exceptions are described below), diluted to the desired concentration with buffer and stored on ice until use. Indomethacin was dissolved in equimolar Na₂CO₃ solution, glibenclamide in 1,2-propylen glycol, and nifedipine in ethanol. Preliminary experiments on HUA demonstrated that the vascular action of BK was unaffected by the solvents used. Likewise, the basal tone of HUA remained unaltered during 30-min treatment with kinin receptor antagonists or specific blockers (except for ouabain, as described below). The experiments with nifedipine and ouabain were performed in a dark room [12, 38]. During the experimental procedure all agents were added directly to the bath in a volume of 0.15 ml and the concentrations given are the final concentrations in the bath solution.

Experimental protocols

At the beginning of each experiment, functional integrity of HUA vascular smooth muscle layer was tested by contracting the artery with K⁺-rich Krebs’ bicarbonate solution, prepared by equimolar substitution of 60 mM NaCl with 60 mM KCl. This course of action was repeated every 20 min until the sustained KCl-induced contraction was obtained that did not differ from the previous two contractions by more than 10%. Further experimentation with a particular HUA was stopped if tachyphylaxis was apparent.

Cumulative concentration-contraction curves for BK (10⁻⁹–10⁻⁶ M) were obtained for rings previously equilibrated at basal tone. The higher concentration of BK has been administered to an organ bath only after the equilibrium response to lower concentration had been produced. One vascular segment has served as a time control and was exposed only to BK, while the other ring from the same artery has been challenged with BK after the 30-min pretreatment with specific blocker or antagonist.

Data analysis

Since a non-receptor-mediated vasoconstriction of the HUA to high-potassium solution is endothelium-independent [38], in our study the contraction induced by each concentration of BK was expressed as a percentage of the maximal contraction (100%) induced by Krebs’ bicarbonate solution with 60 mM KCl, and was used in the construction of the concentration-response curves. The concentration of BK producing 50% of its own maximum response (EC₅₀) was determined for each curve by using a non-linear least square fitting procedure of the individual experimental data, and was presented as pD₂ value (pD₂ = –log EC₅₀).

The pA₂ value (–log molar concentration of antagonist reducing the agonist response by factor two) for HOE 140 (a kinin B₂ receptor antagonist) was determined from the Schild plot [6, 14] by using BK as an agonist. The concentration ratios (the ratio of the EC₅₀ value for BK in the presence and the absence of antagonist) at different antagonist concentrations were calculated for each experiment. Thus, the mean concentration ratios for BK/HOE 140 pairs were plotted in a Schild diagram using regression analysis, and pA₂ was obtained from the intercept of the regression line with the abscissa. The significance of the Schild plot linearity was tested by analysis of variance. The closeness of the slope to unity was verified by the Student’s t-test, and was considered not different from unity if p > 0.05.

The results are expressed as the means ± standard error of the means (SEM); n refers to the number of experiments. The statistical significance of differences between two means was determined with Student’s t-test. A value of p < 0.05 was considered to be statistically significant. All calculations were done by
using the computer program Graph Pad Prism (Graph Pad Software Inc., San Diego, USA).

Results

BK (10^{-9}–10^{-6} M) produced concentration-dependent contraction of HUA obtained from women with normal, hypertensive or diabetic pregnancy (Tab. 1, Fig. 1A). The concentration-response curve for BK was significantly shifted to the left versus the control response only for HUA from PIH, (p < 0.05) (Tab. 1, Fig. 1A).

After endothelial denudation, vascular responses to BK were unaltered versus the respective controls (Tab. 1, Fig. 1). Only in GDM concentration-contraction curve for BK was significantly shifted to the left in relation to the control curve in normal pregnancy (p < 0.05, Tab. 1). L-NOARG (10^{-5} M), an inhibitor of nitric oxide (NO) synthase, did not affect BK-evoked HUA contraction from any of the groups of women (p > 0.05, Tab. 1).

Indomethacin (10^{-5} M), a cyclooxygenase inhibitor, significantly (p < 0.05) reduced BK-produced HUA contraction obtained after the delivery from women both with normal and pathological pregnancy (Tab. 1, Fig. 1). OKY-046 (10^{-5} M), an inhibitor of thromboxane A_2-synthase, significantly (p < 0.05) shifted concentration-contraction curve for BK to the right in PIH, whereas notable (p < 0.01) reduction in maximal contractile response was obtained in HUA from women with GDM (Tab. 1, Fig. 1).

Pretreatment with 10^{-7} M nifedipine (the voltage-gated L-type Ca^{2+} channel blocker) significantly (p < 0.05) inhibited BK-produced maximal contraction in all investigated groups (Tab. 1, Fig. 1). Nifedipine-evoked reduction was particularly evident in arteries from women with PIH.

In all investigated groups, the administration of ouabain (an inhibitor of Na^+/K^+-ATPase, 10^{-4} M) produced contraction of vascular rings equilibrated at basal tone. Thus, in HUA from normal pregnancies, ouabain increased the basal tone to 107.2 ± 11.2% comparing to the reference KCl (60 mM)-induced contraction, whereas in PIH and GDM the basal tone was only enhanced to the point of 80.0 ± 9.1% and 52.7 ± 4.9%, respectively (n = 6–7). Still, maximal contractions obtained after the subsequent addition of BK were unaltered in uncomplicated pregnancy (140.1 ± 6.3%), PIH (135.2 ± 10.2%) and GDM (156.0 ± 9.7%), comparing with those in relevant controls.

| Tab. 1. Contractile effect of bradykinin (BK) in isolated human umbilical artery before and after endothelial denudation or incubation with L-NOARG, indomethacin, OKY-046, glibenclamide, TEA, Ba^{2+}, apamin, nifedipine or des-Arg^9(leu^7)-BK |
|---------------------------------|---------------------------------|---------------------------------|
|                                | Normal pregnancy                | Pregnancy-induced hypertension  | Gestational diabetes mellitus  |
|                                | pEC_{50} ± SEM | max (%) ± SEM       | pEC_{50} ± SEM | max (%) ± SEM       | pEC_{50} ± SEM | max (%) ± SEM       |
| Control                        | 7.51 ± 0.04 | 165.6 ± 4.1          | 8.03 ± 0.14# | 152.4 ± 6.4          | 7.67 ± 0.15 | 152.8 ± 3.1          |
| Endothelium (–)                | 7.21 ± 0.06 | 155.6 ± 14.2         | 7.57 ± 0.05  | 153.1 ± 15.5         | 8.08 ± 0.05# | 163.0 ± 9.5          |
| L-NOARG, 10^{-5} M             | 7.48 ± 0.03 | 168.3 ± 17.8         | 7.94 ± 0.05  | 159.8 ± 13.6         | 7.72 ± 0.19 | 163.5 ± 16.8         |
| Indomethacin, 10^{-5} M        | 6.49 ± 0.12* | 126.2 ± 15.4*       | 7.19 ± 0.08* | 101.2 ± 12.2*#       | 6.48 ± 0.25*# | 131.4 ± 11.3*#       |
| OKY-046, 10^{-5} M             | 7.69 ± 0.06 | 170.8 ± 15.4         | 6.59 ± 0.12*# | 150.6 ± 10.3         | 7.31 ± 0.12 | 97.7 ± 9.3*#         |
| Nifedipine, 10^{-5} M          | 7.63 ± 0.07 | 123.6 ± 15.3*        | 6.83 ± 0.16* | 55.8 ± 6.5*#         | 8.44 ± 0.18# | 107.7 ± 9.4*#        |
| Glibenclamide, 10^{-5} M       | 7.66 ± 0.04 | 159.6 ± 15.9         | 8.02 ± 0.02  | 168.7 ± 10.6         | 7.64 ± 0.13 | 144.6 ± 15.9         |
| TEA, 5 × 10^{-4} M             | 7.41 ± 0.05 | 149.3 ± 7.3          | 7.96 ± 0.2   | 189.1 ± 8.1*#        | 7.45 ± 0.05 | 139.8 ± 7.1          |
| Ba^{2+}, 3 × 10^{-5} M         | 7.36 ± 0.04 | 169.3 ± 8.1          | 8.21 ± 0.02  | 137.4 ± 4.9          | 7.50 ± 0.10 | 182.8 ± 20.7*#       |
| Apamin, 2 × 10^{-5} M          | 7.30 ± 0.1  | 184.3 ± 5.9*         | 7.71 ± 1.0   | 141.3 ± 7.3          | 7.59 ± 0.11 | 137.4 ± 13.9         |
| des-Arg^9(leu^7)-BK, 10^{-5} M | 7.36 ± 0.11 | 159.4 ± 13.9         | 7.93 ± 0.21  | 157.0 ± 20.1         | 7.59 ± 0.09 | 157.3 ± 15.9         |

*p < 0.05 compared to the respective control, #p < 0.05 compared to the normal pregnancy control, n = 5–7
In the next part of our study, we have investigated the effect of several potassium channel blockers, namely glibenclamide (an ATP-sensitive K⁺ channel blocker), tetraethylammonium bromide (a non-selective K⁺ channel blocker), BaCl₂ (an inwardly rectifying K⁺ channel blocker) and apamin (the small conductance Ca²⁺-activated K⁺ channel blocker). Summing up only the positive results, in vascular preparations from uncomplicated pregnancy, PIH and GDM, maximal control contraction produced by BK was notably \( p < 0.05 \) enhanced only for \( 2 \times 10^{-6} \) M apamin, \( 5 \times 10^{-4} \) M TEA, and \( 3 \times 10^{-6} \) M Ba⁺⁺, respectively (Tab. 1, Fig. 1B–D).

Des-Arg⁹(leu⁸)-bradykinin (10⁻⁵ M), a kinin B₁ receptor antagonist, did not affect \( p > 0.05 \) BK-evoked contraction in normal pregnancy, nor did it in PIH and GDM (Tab. 1).

Fig. 1. The effect of bradykinin in human umbilical artery obtained from women with normal pregnancy, pregnancy-induced hypertension and gestational diabetes mellitus (A). Panels B–D represent bradykinin-induced contraction in the absence or presence of different pharmacological blockers. Each point represents the mean ± SEM \((n = 5–7)\). Responses are expressed as percentages of the maximal contraction induced by Krebs' bicarbonate solution with 60 mM KCl, \((n = \text{number of vessels})\).
Human umbilical artery response to bradykinin in pregnancy

9.0 8.5 8.0 7.5 7.0 6.5 6.0 9.0 8.5 8.0 7.5 7.0 6.5 6.0

Fig. 2. The antagonistic effect of HOE 140 (10^{-6}–6 \times 10^{-7} M) on bradykinin-induced contraction of human umbilical artery obtained from women with normal pregnancy (A), pregnancy-induced hypertension (B), and gestational diabetes mellitus (C). Each point represents the mean ± SEM (n = 5–7). Responses are expressed as percentages of the maximal contraction induced by Krebs’ bicarbonate solution with 60 mM KCl. Panel D represents Schild plot of log(concentration ratio–1) vs. −log[antagonist] for bradykinin/HOE 140 antagonism on umbilical artery. Each point represents the mean (n = 5–7). The intercept on the abscissa scale gives pA_{2} value for HOE 140.

Tab. 2. Contractile effect of bradykinin in isolated human umbilical artery in the presence of increasing concentrations of HOE 140, a selective antagonist of kinin B_{2} receptors

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<thead>
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<th>Normal pregnancy</th>
<th>Pregnancy-induced hypertension</th>
<th>Gestational diabetes mellitus</th>
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<tbody>
<tr>
<td></td>
<td>pEC_{50} ± SEM</td>
<td>max (%) ± SEM</td>
<td>pEC_{50} ± SEM</td>
</tr>
<tr>
<td>Control</td>
<td>7.62 ± 0.11</td>
<td>151.4 ± 12.2</td>
<td>7.92 ± 0.06</td>
</tr>
<tr>
<td>HOE 140, 1 \times 10^{-6} M</td>
<td>6.64 ± 0.16</td>
<td>140.0 ± 8.1</td>
<td>6.77 ± 0.17</td>
</tr>
<tr>
<td>HOE 140, 3 \times 10^{-7} M</td>
<td>6.31 ± 0.06</td>
<td>115.4 ± 8.7</td>
<td>6.35 ± 0.08</td>
</tr>
<tr>
<td>HOE 140, 6 \times 10^{-7} M</td>
<td>6.01 ± 0.25</td>
<td>120.0 ± 9.3</td>
<td>6.29 ± 0.04</td>
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In the final part of our study, HOE 140 (10^{-7} - 10^{-6} \times 10^{-7} \text{ M}), a selective kinin B2 receptor antagonist, produced concentration-dependent inhibition of BK-evoked contraction in all investigated groups (Tab. 2; Fig. 2A–C). The data from this study with kinin B2 receptor antagonist were analyzed as described by Arunlakshana and Schild [6] and Kenakin [14]. Thus, the experiments with HOE 140 yielded a straight line with the following mean slopes of the Schild plot:

- **Normal pregnancy**: $0.877 \pm 0.065$
- **PIH**: $1.102 \pm 0.076$
- **GDM**: $0.714 \pm 0.119$

The coefficient of correlation was 0.997, 0.996, and 0.986 in uncomplicated pregnancy, PIH, and GDM, respectively.

### Discussion

**BK** is a vasoactive kinin that is present in fetal circulation and has an important role in local regulation of vascular tone in the placental and umbilical blood vessels, as well as in postpartum closing of ductus arteriosus [2, 3, 11]. Nevertheless, the mechanisms mediating the vascular actions of BK in normal and pathological pregnancy require clarification.

In our study, BK-induced concentration-dependent contraction of intact umbilical arteries in all studied groups of women. Analysis of the pEC_{50} values indicate a significant potentiation of vasoconstriction in HUA from PIH. Thus, vascular sensitivity to BK is likely to be enhanced in hypertensive pregnancy.

Maximal vascular response of umbilical artery rings to BK was generally unaltered after endothelial denudation. Still, according to the obtained results it may be proposed that in diabetic pregnancy functional integrity of endothelium most probably provides certain protective mechanism by modulating the contractile effects of BK, and perhaps of other vasoconstrictors, as well. NO synthase inhibition did not alter vascular response of umbilical artery to BK in any of the investigated groups, suggesting that nitric oxide synthesis does not modulate the contractile effects of BK. Similar results have been also obtained with another NOS inhibitor, N^G\text{-nitro-L-arginine} [16]. This finding may also be linked to the observation that the sensitivity of HUA smooth muscle to endothelial nitric oxide gradually decreases with the progress of pregnancy [13].

In the normal pregnancy, PIH, and GDM, indomethacin-induced cyclooxygenase inhibition significantly reduced the vasoconstrictor response of the HUA to BK. This was not the case in the investigation of Tiritilli et al. [33]. However, we also found a significant reduction in vascular sensitivity to the effects of BK, as well as decrease in BK-induced maximal contraction after inhibition of thromboxane A2 synthase in tissues from PIH and GDM, respectively. Taking into account the conditions required for the activation of cyclooxygenase in HUA [7, 25], our findings are comparable to the results of Amarnani et al. [3]. Namely, it was reported that indomethacin reduced BK-elicited contraction and consequently increased the perfusion pressure in the vasculature of isolated human placental cotyledones. Our results are also consistent with the fact that the balance between prostacyclin and thromboxane A2 is shifted in an unfavorable direction in pregnancies complicated by hypertension [35], with higher levels of thromboxane A2 measured in placentas from preeclamptic pregnancies [31, 36]. In consideration to GDM, Saldeen et al. [30] demonstrated that in diabetic (or impaired glucose tolerance) pregnancy the umbilical artery pulsatility index was positively correlated to the prostacyclin/thromboxane A2 ratio in the cord vessel segments. It was additionally confirmed that during diabetes the ischemic placental lesions were also associated with a high thromboxane A2 in the cord plasma [29]. Furthermore, taking into account our result in GDM obtained after the endothelial denudation of HUA, as well as previous finding about 8-isoprostaglandin F2 production by HUA endothelial cells in normoxia, hypoxia and reoxygenation [37], the contribution of the quoted prostaglandin to BK action should not be excluded.

A key physiological event in the vascular smooth muscle cell contraction is an increase in intracellular calcium concentration *via* either the release from intracellular stores and/or from influx from the extracellular space [15, 20]. In our studies with HUA, nifedipine significantly inhibited BK-induced contraction in all investigated groups, but with significantly greater action in PIH. Our data thus indicate that voltage-gated Ca^{2+} channels significantly contribute to the overall BK-mediated vasoconstriction in the umbili-
the final calculated pA₂ values were 8.106, 8.085 and 7.892, respectively. These pA₂ values for HOE 140 antagonism in HUA are comparable to those obtained in other tissues indicating the involvement of kinin B₂ receptors in BK action [26]. Moreover, they were also similar to that obtained for HOE 140 by Félétou et al. [10] on umbilical artery from uncomplicated pregnancy under the same experimental conditions (8.16 ± 0.16), although slightly higher than the pA₂ value from the experiments of Abbas et al. [1] (7.45–7.62). These data suggest that in normal, hypertensive and diabetic pregnancy BK and HOE 140 interact with kinin B₂ receptors. In addition, based on the obtained mean slope of the Schild plot different from unity, the effects of BK in GDM may involve additional mechanisms.

In conclusion, in normal pregnancy, PIH and GDM bradykinin produced concentration-dependent contraction of HUA via the activation of kinin B₂ receptors. Intact endothelium provided additional modulation of BK contraction in GDM. Cyclooxygenase contractile products significantly participated in BK transduction mechanism, and in PIH and GDM thromboxane A₂ was involved. Voltage-gated Ca²⁺ channels and Na⁺/K⁺-ATPase contribute to the BK-evoked contraction, as well as to the regulation of the basal vascular tone, respectively. Potassium channels modulate BK-induced contraction in human umbilical artery by limiting the excessive vasoconstriction of the investigated kinin.

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