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Altered response of human umbilical artery to 5-HT in gestational diabetic pregnancy

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Abstract:
The aim of this investigation was to evaluate serotonin (5-HT) action on isolated human umbilical arteries (HUA) from normal and gestational diabetes mellitus (GDM) pregnancies. 5-HT caused HUA contraction in a concentration-dependent manner in both investigated groups but with lower efficacy in GDM. After endothelial denudation or in the presence of indomethacin (cyclooxygenase inhibitor), the 5-HT-evoked response was comparably augmented, but only in arteries from uncomplicated pregnancies. 5-HT contractions were unchanged by L-NOARG (NO-synthase inhibitor) or glibenclamide (K_{ATP} channel blocker) in both investigated groups. Whereas nifedipine (Ca^{2+} channel blocker) reduced the contractile effect of 5-HT and was more potent in GDM, ouabain (Na^+/K^+-ATPase inhibitor) caused the contraction of HUA prior to 5-HT addition in both groups, but with a significantly reduced effect in GDM. In vascular rings from GDM, methiothepin (a 5-HT_1/5-HT_2 receptor antagonist) significantly reduced 5-HT-induced contraction to a similar extent as compared to uncomplicated pregnancies. Ketanserin (a 5-HT_{2A} receptor antagonist) produced a concentration-dependent inhibition of the 5-HT effect in GDM. In conclusion, in normal pregnancies, 5-HT produced a concentration- and endothelium-dependent contraction of HUA, most probably *via* endothelial prostacyclin. In contrast, the contractile effect of 5-HT in GDM was reduced with apparent endothelial dysfunction. In both normal and diabetic pregnancies, voltage-gated Ca^{2+} channels and Na^+/K^+-ATPase contribute to the 5-HT-evoked contraction, as well to the regulation of basal vascular tone, but those actions were notably impaired in GDM. In uncomplicated and diabetic pregnancies, the transduction mechanism of 5-HT involves activation of mixed population of 5-HT_1 and 5-HT_{2A} receptors in the HUA.

Key words:
5-HT, human umbilical artery, endothelium, gestational diabetes mellitus

Introduction

The umbilical artery is a crucial component of fetoplacental circulation with the vital function of providing suitable perfusion and nutrition for the placenta [5, 14]. Umbilical blood vessels have been shown to be deficient in autonomic innervation, and for this reason, fetoplacental blood flow is predominantly influenced by the action of local autocrine vasoactive substances, or those provided through the systemic circulation. Serotonin (5-HT) is important vasoactive agent that contributes to the physiological vasomotor function of the human umbilical artery [9]. In fact,

5-HT is potent vasoconstrictor of human umbilical blood vessels. Although seven main groups of 5-HT receptors (5-HT_1–5-HT_7) have been characterized, it has been established that 5-HT_1 and 5-HT_2 receptor subtypes are involved in contractile responses of human umbilical arteries from uncomplicated or preeclamptic pregnancies [8, 10, 13].

Gestational diabetes mellitus (GDM) is commonly identified as any degree of glucose intolerance that begins or is first recognized during pregnancy [3]. It is well established that increased risks of adverse fetal outcomes are present if GDM is not treated adequately [21]. For example, these include macrosomia, fetal distress, intrauterine growth restriction or stillbirth. A very important and common feature of diabetic disorders is dysfunction of endothelial cells at various levels [6]. Moreover, endothelial dysfunction during diabetic pregnancies may be associated with higher risks for concurrent hypertensive disorders during pregnancy [16]. Likewise, impaired endothelium-dependent vasodilatation was previously documented in women with prior gestational diabetes [2]. Thus, these observations suggest that the response of the human umbilical artery to different vasoactive substances, including 5-HT, may be altered in GDM.

Considering that 5-HT-produced effects in HUA from GDM are not fully known, the present experiments were undertaken in order (1) to relate the effect of 5-HT on isolated HUA obtained from women with uncomplicated pregnancies and GDM, (2) to evaluate the role of the vascular endothelium and the possible input of endothelial factors in 5-HT-evoked artery responses in physiological and pathological contexts, (3) to assess the contribution of extracellular and intracellular calcium in the effect of 5-HT in both investigated groups and (4) to determine the receptor population that mediates the action of 5-HT on HUA.

Materials and Methods

Preparation of human umbilical artery

The experiments were performed on human umbilical arteries isolated from the middle part of umbilical cords obtained immediately after vaginal delivery from women with normal pregnancies and after Cesarean section in women with pathological pregnancies. Only the remnant tissues, which would have been otherwise disposed, were utilized [18]. Our investigation enrolled 23 women with normal, full-term pregnancies (middle maternal age = 26) and 11 women with gestational diabetes mellitus (middle maternal age = 31). The presence of glucose intolerance was tested in pregnant woman without known diabetes by a glucose challenge test, and gestational diabetes mellitus was additionally confirmed by a standard oral glucose tolerance test [3]. All women were hospitalized and followed-up at the Institute of Obstetrics and Gynecology, Medical Faculty, Belgrade. The present study was approved by the Ethics Committee at the Medical Faculty, Belgrade.

The obtained umbilical cords were kept overnight at 4°C, and experiments on umbilical arteries were carried out within 24 h after delivery [2, 24]. At the beginning of each experiment, umbilical arteries were isolated from the Wharton 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 were prepared. These were randomly allocated for experiments using different pharmacological blockers or receptor antagonists. The endothelium was removed from some of the rings by gently rubbing the intimal surface with a stainless-steel wire [18]. Ring preparations were mounted between two stainless-steel triangles in an organ bath containing 20 ml of Krebs' bicarbonate solution (37°C, pH 7.4), aerated with 95% O_2 and 5% CO_2. One of the triangles was attached to a displacement unit to allow for fine adjustment of tension, and it was 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 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 over the next 30 min to the resting tension of 2 g ≈ 20 mN [1] and additionally equilibrated for 30 min before experimentation.

Drugs and solutions

The Krebs' bicarbonate solution used for this study was composed as follows (in mM): NaCl 115.0; KCl 4.6; $CaCl_2$ 2.5; $MgSO_4$ 2.5; KH_2PO_4 1.2; $NaHCO_3$

25.0; glucose 11.1. The following drugs were used: 5-HT, methiothepin, ketanserin, nifedipine (ICN, Irvine, CA, USA), indomethacin, glibenclamide, ouabain (Sigma, St. Louis, USA), and N^G-nitro-L-arginine (L-NOARG) (RBI, Natick, MA, USA). All reagents were dissolved in distilled water (except as described below), diluted to the desired concentration with buffer and stored on ice until use. Indomethacin was dissolved in an equimolar Na_2CO_3 solution, glibenclamide in 1,2-propylene glycol, and nifedipine in ethanol. Preliminary experiments in HUA demonstrated that the vascular action of 5-HT was unaffected by the solvents used. Likewise, the basal tone of HUA remained unaltered during 30-min treatment with the blockers (except for ouabain, as described below). Experiments with nifedipine and ouabain were performed in a dark room [17]. All agents were added directly to the bath in a volume of 0.15 ml, and the concentrations presented in this work reflect the calculated final concentrations in the bath solution.

Experimental protocols

At the beginning of each experiment, the functional integrity of the vascular smooth muscle layer of the HUA 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 a 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 5-HT (10^{-9}–3×10^{-5} M) were obtained in rings previously equilibrated at basal tone. The higher concentration of agonist was administered in an organ bath only after the equilibrium response at the lower concentration was produced. One vascular segment served as the time control and was exposed only to 5-HT, while the other ring from the same artery was challenged with 5-HT after 30-min pretreatment with a specific blocker or antagonist.

Data analysis

Contraction induced by each concentration of 5-HT was expressed as a percentage of the maximal contraction (100%) induced by Krebs' bicarbonate solution with 60 mM KCl [19] and was fitted to construct concentration-response curves. The concentration of 5-HT producing 50% of its own maximum response (EC_{50}) was determined for each curve by using a non-linear least square fitting procedure of the individual experimental data and was presented as pEC_{50} value ($pEC_{50} = -\log EC_{50}$).

The pA_2 value (–log molar concentration of antagonist reducing the agonist response by a factor of two) for ketanserin (a $5\text{-}HT_{2A}$ receptor antagonist) was determined from the Schild plot [4, 11] using 5-HT as an agonist. The concentration ratios (the ratio of the EC_{50} value for 5-HT in the presence and absence of antagonist) at different antagonist concentrations were calculated for each experiment. The mean concentration ratios for 5-HT/ketanserin pairs were plotted in a Schild diagram using regression analysis, and pA_2 was obtained from the intercept of the regres-

Tab. 1. Contractile effect of serotonin (5-HT) in isolated human umbilical artery in normal pregnancies and gestational diabetes mellitus.

	Normal pregnancy		Gestational diabetes mellitus	
	pEC_{50} ± SEM	max (%) ± SEM	pEC_{50} ± SEM	max (%) ± SEM
Control	6.69 ± 0.02	209.9 ± 15.4	7.24 ± 0.04	169.8 ± 19.3
Endothelium (–)	6.50 ± 0.04	250.7 ± 14.3*	7.19 ± 0.09	178.8 ± 12.7
L-NOARG, 10^{-5} M	6.88 ± 0.03	230.3 ± 14.2	7.36 ± 0.07	149.3 ± 15.9
Indomethacin, 10^{-5} M	6.47 ± 0.03	256.9 ± 12.5*	7.47 ± 0.03	159.6 ± 18.0
Glibenclamide, 10^{-6} M	6.99 ± 0.03	230.6 ± 7.3	6.90 ± 0.06	149.1 ± 14.3
Nifedipine, 10^{-7} M	6.55 ± 0.04	186.1 ± 10.0*	6.27 ± 0.03#	140.1 ± 13.8#
Methiothepin, 10^{-7} M	6.48 ± 0.01	156.4 ± 15.1*	7.14 ± 0.03	124.5 ± 11.6#

* $p < 0.05$ and # $p < 0.05$ compared to control values from normal pregnancies and GDM, respectively, n = 5–7

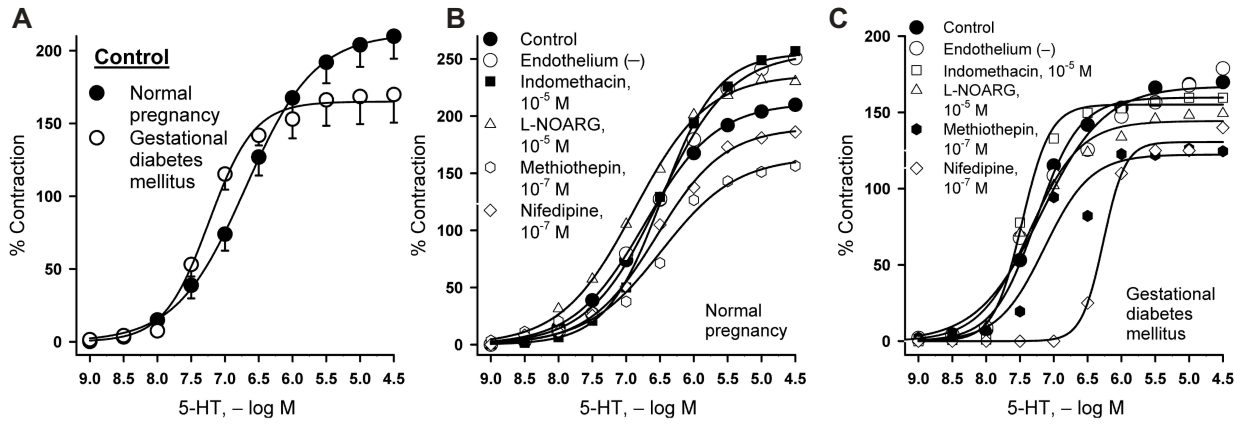

Fig. 1. The effect of 5-HT on human umbilical arteries obtained from women with normal pregnancies and gestational diabetes mellitus (**A**). Panels **B** and **C** represent 5-HT-induced contraction in the absence or presence of different pharmacological blockers. Each point represents the mean ± SEM (n = 5–7). SE from panels **B** and **C** were excluded for better clarity. Responses are expressed as percentages of the maximal contraction induced by Krebs' bicarbonate solution with 60 mM KCl

sion line with the abscissa. The significance of the Schild plot linearity was tested by an 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 the Student's *t*-test. A value of $p < 0.05$ was considered to be statistically significant.

Results

5-HT (10^{-9}–3×10^{-5} M) produced concentration-dependent contraction of intact HUA in all investigated groups (Tab. 1, Fig. 1A). When compared with uncomplicated pregnancies, the concentration-response curve for 5-HT in gestational diabetes mellitus was shifted to the left with a concomitant and significant reduction of the maximal artery response ($p < 0.05$).

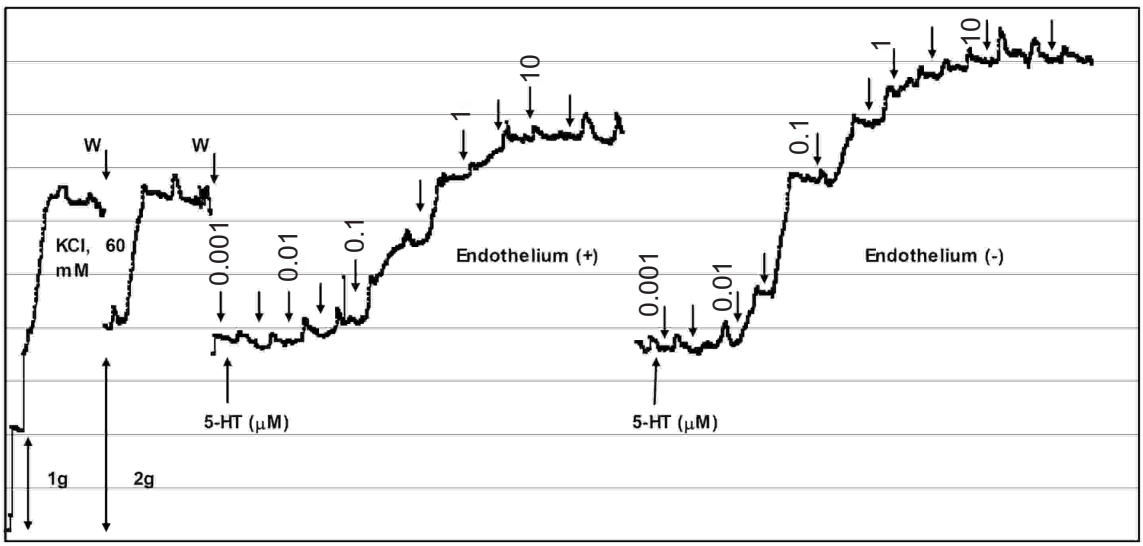

Fig. 2. Original recordings of 5-HT-produced cumulative vascular response of human umbilical artery obtained from women with uncomplicated pregnancies. One vascular segment served as a time control (intact artery), while the other ring from the same artery was challenged with 5-HT after endothelial denudation

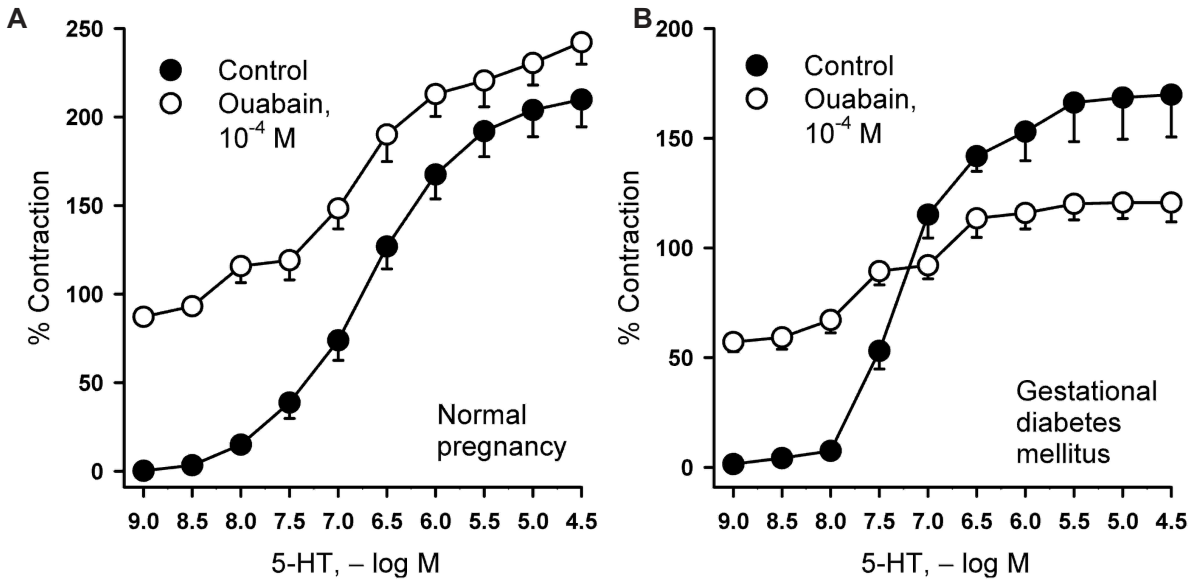

Fig. 3. The effect of 5-HT in human umbilical artery obtained from women with normal pregnancies (**A**) and gestational diabetes mellitus (**B**) in the absence or presence of ouabain (10^{-4} M). Each point represents the mean ± SEM (n = 5–7). Responses are expressed as percentages of the maximal contraction induced by Krebs' bicarbonate solution with 60 mM KCl

After endothelial denudation or incubation with indomethacin (a cyclooxygenase inhibitor, 10^{-5} M), vascular responses to 5-HT in normal pregnancies were significantly and comparably augmented ($p < 0.05$), while unaltered in GDM *versus* respective controls (Tab. 1, Fig. 1B-C). Figure 2 shows original recordings of 5-HT-produced vascular responses of HUA with and without endothelium obtained from women with uncomplicated pregnancies.

L-NOARG (10^{-5} M), an inhibitor of nitric oxide synthase, slightly but not significantly enhanced 5-HT-evoked contraction in HUA from normal pregnancies and at the same time reduced the effect of 5-HT in GDM to the same extent ($p > 0.05$, Tab. 1, Fig. 1B–C).Glibenclamide (an ATP-sensitive K^+ channel blocker, 10^{-6} M) did not affect ($p > 0.05$) 5-HT-evoked contractions in normal or GDM pregnancies (Tab. 1).

Pretreatment with 10^{-7} M nifedipine (the voltage-gated L-type Ca^{2+} channel blocker) significantly inhibited 5-HT-produced contractions in all investigated groups (Tab. 1, Fig. 1B–C, $p < 0.05$), and nifedipine-evoked reduction was more apparent in vascular rings from GDM ($p < 0.05$). Administration of ouabain (an inhibitor of Na^+/K^+-ATPase, 10^{-4} M) produced contractions in arteries equilibrated on basal tone (Fig. 3). In HUA from normal pregnancies, ouabain induced 87.16 ± 8.2% of the referent KCl (60 mM)-induced contraction, and in the group with GDM basal tone was elevated to the point of 57.1 ± 4.4% (n = 7). Maximal contractions obtained after the subsequent addition of 5-HT (10^{-9}–3×10^{-5} M) were enhanced in uncomplicated pregnancies (242.3 ± 12.5%, $p < 0.05$), while they were reduced in GDM (120.7 ± 8.8 %, $p < 0.05$), as compared to respective controls.

Methiothepin (10^{-7} M), a 5-HT_1/5-HT_2 receptor antagonist, significantly reduced 5-HT-evoked contractions in both investigated groups (Tab. 1, Fig. 1B–C, $p < 0.05$). A similar result was obtained in normal pregnancy samples with 10^{-7} M ketanserin, a 5-HT_{2A} receptor antagonist (control – pEC_{50} ± SEM = 6.69 ± 0.02, max. contraction = 209.9 ± 15.4 %; ketanserin 10^{-7} M – pEC_{50} ± SEM = 5.91 ± 0.04, max. contraction = 172.1 ± 14.1 %, $p < 0.05$ *vs*. control). Additional experiments in HUA from GDM have shown that ketanserin (3×10^{-8}–3×10^{-7} M) significantly reduces the 5-HT-induced effect in a concentration-dependent manner (Fig. 4A), with the following results: control – pEC_{50} ± SEM = 7.24 ± 0.04, max. contraction = 169.8 ± 19.3%; ketanserin 3×10^{-8} M – pEC_{50} ± SEM = 6.39 ± 0.15, max. contraction = 153.3 ± 8.8% ($p > 0.05$ *vs*. control); ketanserin 10^{-7} M – pEC_{50} ± SEM = 6.21 ± 0.05, max. contraction = 143.3 ± 15.8% ($p < 0.05$ *vs*. control); ketanserin 3×10^{-7} M – pEC_{50} ± SEM = 5.59 ± 0.25, max. contraction = 134.4 ±

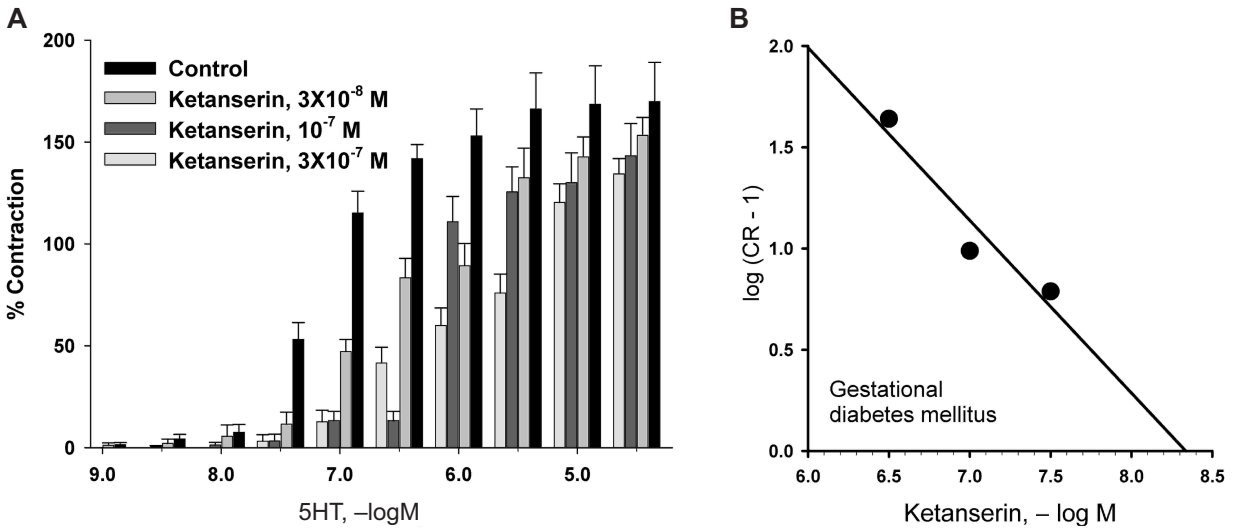

Fig. 4. The antagonism of ketanserin (3×10^{-8}–3×10^{-7} M) on 5-HT-induced contraction in human umbilical artery obtained from women with gestational diabetes mellitus (**A**). Each point represents the mean ± SEM (n = 5–7). Responses are expressed as percentages of the maximal contraction induced by Krebs' bicarbonate solution with 60 mM KCl. Panel **B** represents the Schild plot of log (concentration ratio – 1) *vs.* –log [antagonist] for 5-HT/ketanserin antagonism on umbilical artery. Each point represents the mean (n = 5–7). The intercept on the abscissa scale gives pA_2 value for ketanserin

7.5% ($p < 0.05$ *vs*. control). The data we obtained with ketanserin were analyzed as described by Arunlakshana and Schild [4] and Kenakin [11]. The experiments with ketanserin yielded a straight line and the mean slope of the Schild plot was 0.8523 ± 0.261 (Fig. 4B). The intercept of the Schild's regression line with the abscissa (pA_2 value) for ketanserin was 8.336 ± 0.787, while the coefficient of correlation was 0.956.

Discussion

The aim of this study was to evaluate serotonin (5-HT) action on isolated human umbilical arteries (HUA) in gestational diabetes mellitus (GDM) as compared to vascular preparations from normal pregnancy. We found that 5-HT induced concentration-dependent contraction of intact umbilical arteries from both study groups. However, in diabetic pregnancies, 5-HT contracted vascular rings with higher potency but with lower efficacy. In general, the concentration-response curve for 5-HT in GDM was characterized by a rapid initial rise phase with a premature and decreased maximal response plateau. These characteristics may indicate certain augmentation of umbilical artery sensitivity to 5-HT in pathological pregnancy conditions. It may also be assumed that the decreased maximal response to 5-HT may be related to GDM-associated disruption and degeneration of the vascular smooth muscle layer in human umbilical arteries [20].

5-HT-evoked vascular responses in umbilical arteries in controls were augmented after endothelial denudation, as previously reported by Klockenbusch et al. [12], but this was not the case in diabetic pregnancies. Thus, these results suggest that the endothelium contributes to continual modulation of vascular response to 5-HT during pregnancies, and this is probably mediated *via* endothelial relaxing factors. On the other hand, the opposite result in umbilical artery from diabetic pregnancy suggests the presence of endothelial dysfunction. Our observed endothelium-independent contraction of vascular preparations in GDM is consistent with an already established relationship between pathological alterations in endothelial cells and diabetes [6]. As with endothelial denudation, a comparable result was obtained after cyclooxygenase inhibition, thus suggesting that in normal pregnancies, endothelial-dependent control of 5HT-induced vasoconstriction is most likely mediated through prostacyclin. This hypothesis is also in accordance with the results of Okatani et al. [15], who utilized tranylcy-

promine, an inhibitor of prostacyclin synthesis. However, the absence of an indomethacin effect in GDM further supports our assumption of endothelial dysfunction in human umbilical arteries.

After the inhibition of NO-synthase in vascular rings isolated from uncomplicated pregnancies, the contractile effect of 5-HT was slightly, but not significantly, enhanced, thus suggesting that endothelial nitric oxide does not play a role in this phenomenon. A similar result was previously obtained after the administration of N-nitro-L-arginine (L-NNA), another NO-synthase inhibitor [22]. In GDM, the 5-HT-induced contraction was reduced in the presence of L-NOARG, but to a smaller extent. It has been shown that the activity of NO-synthase in homogenates of umbilical arteries in normal and diabetic pregnancies is not different [7], which indirectly supports our result with L-NOARG in a pathological setting. In addition, glibenclamide did not alter the effect of 5-HT. This suggests that at least in normal pregnancies, endothelium-derived hyperpolarizing factor does not contribute to vasoconstriction produced by 5-HT *via* ATP-sensitive K^+ channels. Likewise, ATP-sensitive K^+ channels do not appear to be involved in the 5-HT pathway in diabetic pregnancies.

The contribution of extracellular calcium on umbilical artery responses to 5-HT was evaluated by pretreatment of vascular preparations with nifedipine, a voltage-gated L-type Ca^{2+} channel blocker. Thus, in uncomplicated pregnancies, we observed a reduction of 5-HT-induced action, which was similar to what was reported by Xie and Triggle [24]. Moreover, it has been shown that the vascular response to 5-HT was also reduced in bath solution without calcium [23, 24]. In our study, the nifedipine-induced inhibition of 5-HT contractions was much more evident in GDM, suggesting enhanced and probably additional pathological involvement of voltage-gated Ca^{2+} channels in transduction mechanisms during human umbilical artery responses to 5-HT.

In our previous study, we showed that the Na^+/K^+-ATPase contributes to bradykinin-evoked contraction of isolated human umbilical arteries [18]. Therefore, the possible involvement of Na^+/K^+-ATPase activity and subsequent contribution of calcium release from intracellular stores was also investigated in 5-HT-evoked contractions. Our results showed that ouabain-induced contractions of vascular rings equilibrated to basal tone were more pronounced in uncomplicated pregnancies, suggesting that the control of intracellular calcium concentration in vascular smooth muscles by the Na^+/K^+-ATPase, and subsequent input to overall basal tone of umbilical arteries is fully functional in normal pregnancies, yet altered in GDM. Accordingly, it has been reported that Na^+/K^+-ATPase activity is reduced in placentas obtained from women with insulin-dependent diabetes mellitus [25]. One additional observation is of significant interest. After subsequent addition of 5-HT on precontracted vessels with ouabain, maximal contractions induced by 5-HT in normal pregnancies were augmented and comparable to those obtained after endothelial denudation or cyclooxygenase inhibition. Thus, it is likely that there is certain synergy between transduction mechanisms involved in 5-HT-induced contractions and the action of Na^+/K^+-ATPase toward maximal vascular responses. However, this observation requires further evaluation.

The aim of the next part of our study was to determine which 5-HT receptors are involved in serotonin-produced contractions in human umbilical arteries, especially given the current lack of data regarding gestational diabetes mellitus. Thus, in normal pregnancies, methiothepin, a nonselective 5-HT_1/5-HT_2 receptor antagonist, significantly reduced 5-HT-induced contraction. Additional experiments with increasing concentrations ($0.01–10 \times 10^{-6}$ M) of methiothepin (data not shown) revealed typical irreversible competitive antagonism, thus confirming that both 5-HT_1 and 5-HT_2 receptors may be involved in 5HT-evoked vascular responses in umbilical arteries. In agreement with quoted finding, previous experiments in normal pregnancies also showed that methiothepin reduced the contractile effects of sumatriptan (5-HT_{1D}/5-HT_{1B} receptor agonist) and alpha-methyl-5-HT (5-HT_2 receptor agonist) [10]. Similar results were reported by Gupta et al. [8]. In vascular preparations from GDM, methiothepin significantly reduced 5-HT-induced contraction. Thus, these observations affirm the contribution of 5-HT_1/5-HT_2 receptors in the action of 5HT in isolated arteries from diabetic pregnancies.

In this study, ketanserin (5-HT_{2A} receptor antagonist) notably decreased 5-HT-induced contractions in vascular rings isolated from uncomplicated pregnancies. The apparent contribution of 5-HT_{2A} receptors in the vascular effect of 5-HT on umbilical arteries from normal pregnancies was reported by Lovren et al. [13]. They also determined pA_2 value for ketanserin's competitive antagonism on 5-HT action to be 8.67 (7.95–9.40). Based on these observations, we ex-

tended our experiments to umbilical arteries from diabetic pregnancies. Thus, increasing concentrations of ketanserin significantly reduced the 5-HT-induced effect in a concentration-dependent manner, and the obtained pA_2 value was 8.336. Although the estimated coefficient of correlation (0.956) and the pA_2 value suggest a major role of 5-HT_{2A} receptors in 5-HT-induced contraction in vascular rings from GDM, a mean slope of the Schild plot different from 1 implies further involvement of other 5-HT receptors in the investigated effect.

In conclusion, in normal pregnancies, 5-HT produces concentration- and endothelium-dependent contraction of the human umbilical artery, in which the maximal vascular response is likely controlled by endothelial prostacyclin. On the other hand, the contractile effect of 5-HT in GDM is reduced with accompanying endothelial dysfunction. Although in both normal and diabetic pregnancies, voltage-gated Ca^{2+} channels and the Na^+/K^+-ATPase contribute to 5-HT-evoked contraction, as well as regulation of basal vascular tone, respectively, these actions are notably impaired in GDM. The transduction mechanism of the umbilical artery response to 5-HT in uncomplicated and diabetic pregnancies involves activation of a mixed population of smooth muscle 5-HT_1 and 5-HT_{2A} receptors.

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