Increased bradykinin B1 receptor expression in diabetic placental tissue correlates with both hyperhistaminaemia and increased histidine decarboxylase (HDC) activity.

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Abstract: Increased histamine content and bradykinin levels were reported in placental tissue obtained after diabetic pregnancies. Both compounds are well known mediators of the inflammatory reactions. Bradykinin induces mast cell degranulation, leading to increase in the local histamine concentration. Bradykinin B1 receptor shows strong involvement in the inflammatory response, whereas the B2 receptor mediates most of the effects induced by kinins. The aim of this study was to examine comparatively correlations between placental HDC activity, histamine concentration, and placental bradykinin receptors B1 and B2 expression in diabetes class C versus normal pregnancy. Sixteen diabetic placentae were compared with 16 normal placentae (Group I and II, respectively). Tissular activity of HDC was assayed in placental samples obtained in a standardized manner, using a modified method, described previously by Endo. Histamine concentrations in placental cuts were estimated fluorimetrically, whereas expression of B1 and B2 was examined in immunostained paraffin sections by quantitative morphometry in the areas matched in mean vascular density. Mean HDC activity in group I was significantly (p < 0.05) increased compared to controls (3.97 ±0.25 vs 2.88 ±0.15 nmol/h/g ±SEM). Histamine concentration in Group I was significantly increased compared to Group II (387 ±25.3 vs 239 ±14.3 ng/g of wet weight ±SEM). Mean expression of the B1 was augmented in diabetes and reached 289.8% of the value for Group II (p<0.05). The differences in mean expression of B2 receptors were non significant. Increased HDC activity may explain particular phenomena observed in diabetic placental tissue. HDC-dependent histamine elevation may change locally vascular properties by influence on bradykinin receptors expression. Proinflammatory reactions mediated via B1 should be expected rather, than changed vasomotor reactivity related to B2. Angiogenic properties of histamine and kinins should also be considered.

Keywords: histidine decarboxylase, histamine concentration, bradykinin receptors, human placenta, diabetes-complicated pregnancy

1 Introduction
Human placental tissue contains a moderate amount of histamine. Histamine is a biogenic amine, derived from the decarboxylation of histidine in reaction catalyzed by L-histidine decarboxylase (HDC; EC 4.1.1.22). Vesicular structures (granules) of mast cells are the main source of placental histamine [1]. Among many other mast cell-derived substances, placental histamine acting via specific histamine receptors is considered as important pro-inflammatory mediator, local regulator of the vascular resistance and permeability as well as the growth (angiogenic) factor [2,3].

The nonapeptide bradykinin is the final product of the kinin system and is split from a serum alpha-2-globulin precursor by the kallikreins (EC 3.4.4.21) and also by trypsin (EC 3.4.4.4) or plasmin (EC 3.4.4.14) [4]. Bradykinin is a potent endothelium-dependent vasodilator for most vessels, except for the placental, causes contraction of non-vascular smooth muscle, increases vascular permeability and also is involved in the mechanism of pain. In some aspects, it has similar actions to that of histamine. Moreover, some amount of bradykinin is present in the granules of mast cells [5]. The kinin B1 and B2 receptors belong to G protein coupled receptor (GPCR) family. Expression of bradykinin B1 receptor becomes evident during inflammatory response, whereas the B2 receptor mediates most of the effects induced by kinins [6].
Diabetes mellitus is one of the most common medical complications of pregnancy. Diabetes evokes a complex series of events that results in structural and functional abnormalities in many tissues and body compartments, including uterofetoplacental unit [7]. For that reason, diabetes in pregnancy can have serious consequences for the mother and the growing fetus. Maternal ketoacidosis and iatrogenic hypoglycemia may be life threatening. Fetuses or infants of mothers with diabetes are at greater risk for several problems, especially if blood glucose levels are not carefully controlled, including the following: birth defects, stillbirth (fetal death), macrosomia, birth injury (due to the baby’s macrosomia), hypoglycemia after delivery and respiratory distress [8]. The placenta of the diabetic patient has attracted much interest largely because it is thought that placental damage may be partially responsible for the unduly high incidence of fetal complications that occur in pregnancies complicated by diabetes mellitus [9]. Class C diabetes in pregnancy (after White) is the last stage without recognized vascular changes in light microscopy [10]. The results of our previous studies showed, that in diabetes class C an increased density of the villous network of microvessels correlates with higher histamine concentration and increased number of mast cells [11,12]. Increased levels of bradykinin have also been reported in diabetes [13]. It was proved that bradykinin induces mast cell degranulation, leading to increase in the local histamine concentration [14].

2 Aim of the study
The aim of the present investigation was to examine comparatively correlations between placental HDC activity, histamine concentration and placental bradykinin receptors B1 and B2 expression in diabetes class C versus normal pregnancy.

3 Materials and methods
This study was conducted in compliance with international and local laws of human experimentation and the project was approved by the local ethics committee. All subjects provided written ethical consent to the collection and use of their tissues, according to the standards published by British Medical Research Council as “Human tissue and use of biological samples: operational and ethical guidance 2001 (includes Addendum to MRC 2001 guidance following the Human Tissue Act 2004)”. Sixteen placentae were obtained after pregnancies complicated by diabetes class C (after White) and gestationally matched with 16 placentae collected after normal-course pregnancies (group I and group II, respectively) [10]. The control of glycemia in all cases was satisfactory: the levels of fraction of glycosylated hemoglobin (HbA1c) in all trimesters of diabetic pregnancy were kept within the normal range (5–7.5%). The gravidas in both groups were nulliparas and the mean gestational age amounted to 252 ± 6 days. All newborns in group I were delivered by elective cesarean sections in fetal interest, while in group II the operative indications were: high myopia or pelvic longitudinal lie of the fetus (breech presentation). Five specimens of placental tissue were excised in a standardized manner: two from the region contiguous to fetal surface of the placenta (the first – from place of umbilical cord insertion, the next – from peripheral region), and three from the region contiguous to maternal surface (the first one – from the central part, the next two – from peripheral regions of the placental maternal surface) (see Fig. 1). The samples obtained after cesarean section were at once frozen in carbon dioxide snow for histamine concentration measurement. Simultaneously, further samples were fixed in formalin, embedded in paraffin wax and cut at 5 µm, before staining with hematoxylin/eosin and immunostainings.

3.2 Assessment of HDC activity
Tissular activity of HDC was assayed in placental samples obtained in a standardized manner. A modified method, described previously by Endo was applied [15]. Briefly, histamine-free enzyme solution containing HDC has been obtained after homogenization with phosphorylated cellulose and centrifugation. Then, newly formed histamine after incubation of the enzyme solution with histidine (1mM) was separated by chromatography and quantified fluorometrically as an indicator of HDC activity.

3.3 Measurement of histamine
Histamine concentrations in placental cuts were estimated using fluorimetric method [16]. Briefly, determination of histamine was based on a precolumn derivatization with o-phthalaldehyde, using reversed phase high-performance liquid chromatography in perchloric acid extracts. A fluorescence detection system was used: excitation was set at 360 nm and emission was read at 455 nm of wavelengths. The detection limit was 250 pg per sample.

3.4 Immunohistochemistry of B1 and B2
In order to visualize bradykinin B1 and B2 receptors in the paraffin sections (N = 72 for each group, for each
type of the receptor staining), a standard immunohistochemical staining procedures were applied. Rabbit bradykinin B1 receptor antibody (B1R; extracellular domain; reacting with Hu, Ms, Rt; dilution of 1:500) and rabbit bradykinin B2 receptor antibody (B2; reacting with Hu, Ms, Rt; dilution of 1:500) were used as the primary antibodies, respectively. Goat anti-rabbit IgG antibodies were used as biotinylated secondary antibody (0.5% v/v). Both, the primary and secondary antibodies were manufactured by Acris Antibodies GmbH, Germany. The visualization of primary anti-receptor antibodies was done using the StreptABCComplex/HRP Duet (Dako Cytomation, Glostrup, Danmark) following the procedure recommended by the producer, where 3,3’-diaminobenzidine served as a chromogen. The negative controls for immunostainings were prepared by replacement of the primary antibodies by normal rabbit preimmune IgG diluted with phosphate buffered saline, containing 3% bovine serum albumin at the same protein concentration as that used for the primary antibodies.

3.5 Mean density of placental microvessels
Assuming, that the accuracy of bradykinin receptors expression measurement may be significantly affected by local differences in density of vascular system, in both groups we examined comparatively vascular density–matched samples (tolerated range of discrepancy was ± 5%) [17]. Mean density of placental microvessels was measured in calibrated areas of the placental paraffinized sections stained with hematoxylin/eosin. Light microscopy with computerized morphometry for quantitative analysis (Quantimet 500+C image analysis system provided by Leica, Cambridge Ltd., Cambridge, UK) was used. The mean Vascular / Extravascular Tissular (V/EVTI) was the main parameter of the vascular density [18,19]. The picture analysis procedure consisted in measurement of the total vascular area. The total lumen area of all types of identified vessels was summed up. In order to eliminate technical error caused by unaxial section of vessel the lowest value of Ferret’s diameter was accepted as the diameter of a single lumen. V/EVTI represents the ratio, which reflects intensity of vascularization. Each preparation underwent three area analyses; the single area measured with the picture analyzer amounted to 721320 µm². The total number of preparations amounted to 288 for each group.

3.6 Expression of B1 and B2
After immunostaining, B1 and B2 receptors expression was estimated using quantitative computerized morphometry (Image analysis system with Quantimet 500+C software by Leica, UK) under the light microscopy [20]. All morphometric procedures were carried out twice by two independent observers, and the average results recorded. Intensity of immunostaining was evaluated using mean colour saturation parameter and thresholdings in grey-level histograms. Expression of bradykinin B1 and B2 corresponded to the total immunostained calibrated area of examined sections, where colour saturation comprises segmentation-separation criteria for objects. Single analysed image area was 721320 µm². In each group 216 visual fields were analysed for each type of bradykinin receptor (3 visual fields per single paraffin slide). In order to assure optimal accuracy of measurements, following factors have been controlled or monitored: illumination, power supply, warming up, shading correction, averaging of image intake, hue, luminance, relation of illumination to quantification of area percentage of positively staining structures.

3.7 Statistical analysis
Mann-Whitney’s U-test was applied. The results were expressed as means ±SEM or mean percentage values ±SEM. Differences between group I (diabetes) and II (normal-course pregnancy) were deemed statistically significant if p < 0.05.

4. Results
Mean HDC activity in group I was significantly (p<0.05) increased compared to controls (3.97 ±0.25 vs 2.88 ±0.15 nmol/h/g ±SEM) as shown in Figure 1. Histamine concentration in diabetes class C (group I) was significantly increased compared to normal controls from group II (387 ±25.3 versus 239 ±14.3 ng/g of wet weight ±SEM); (see Fig. 2B).

The expressions of bradykinin receptors B1 and B2 are presented in Figure 2A. Mean expression of the B1 was augmented in diabetes and reached 289.8% of the value for group II (p<0.05). The differences in mean expression of B2 receptors were non significant.

5. Discussion
The placenta is a complex and still poorly understood organ, which plays the central metabolic role in pregnancy. In addition to synthesizing various hormones it regulates the transport of maternal fuels to the fetus and facilitates maternal metabolic adaptations to different stages of pregnancy [21]. Considering its central position within uterofetoplacental unit, the placenta is exposed to metabolic endocrine and/or perfusion derangements. It is very likely, that diabetic
environment influences receptors, transporters, ion channels and other molecules on both placental surfaces, being in contact with both maternal syncytiotrophoblast) and fetal (endothelium) circulation [9]. The crucial question is still open: whether the placenta adapts to the diabetic environment with the ultimate result of protecting the fetus from the adverse diabetic environment or whether placental changes are responsible for pathophysiology of the fetus and adverse fetal outcome, despite improvement in the care of diabetic women.

Clinical observations and histopathological studies of the placenta have confirmed an increased incidence of inflammatory reactions in diabetes [22]. Based on neutral protease composition, placental mast cell heterogeneity in diabetes class C may also be important. Previously, we reported the shift of the quantitative balance in mast cell tryptase-positive/chymase negative/tryptase positive-chymase positive subtypes towards increase of the last mentioned, and increased total number of placental mast cells [23]. According to Welle’s observation, tryptase seems to participate in pro-inflammatory mast cell function, whereas chymase seems to be more involved in inflammatory reactions [24]. Moreover, knowing that bradykinin levels in diabetes may be elevated and that bradykinin degranulates mast cells, a chronic pro-

Figure 1.
Mean activity of histidine decarboxylase (HDC) in placental tissue after elective cesarean section: diabetes versus non-complicated gestationally matched pregnancies. The mean gestational age amounted to 252 ± 6 days.

Figure 2.
Diabetes White class C (Group I) vs normal course of pregnancy (Group II): correlation between the mean expression of bradykinin receptors B1 and B2 (A.) and the mean histamine concentration within placental tissue (B.). *Mean values obtained for normal pregnancy (0.022 ±0.0017 for B1, and 0.019 ±0.0013 for B2; abstract numbers ±SEM) were taken as 100%.
inflammatory conditions should be suspected within diabetic placenta [13,14]. Degranulation of mast cells will produce additional local increase in bradykinin level. Thus, hyperhistaminemia in diabetic placenta, supported by HDC hyperactivity, may change vascular properties, influencing bradykinin B1 receptor. This mechanism of vicious circle may be responsible for some complications reported in diabetic pregnancy. For example, it should be evaluated if increased rate of major birth defects in infants of diabetic mothers is related to mitogenic properties of bradykinin acting via B1 receptor [25].

Augmented expression of bradykinin B1 receptors without significant changes in B2 expression observed in our study may also suggest pro-inflammatory changes. According to the results of independent authors, B1 receptors are not present in any significant amount in normal tissues and their expression is often inducible rather than constitutive. Kinin B1 receptor-mediated responses are upregulated in a time- and protein production-dependent process [26]. Some experimental data suggest that diabetes is a pathological condition that could induce B1 expression. Insulin-dependent diabetes often derives from an autoimmune response, implicating an overproduction of cytokines such as IL-1β and TNF-α. Moreover, hyperglycemia and oxidative stress can also activate NF-κB. The addition of cytokines overproduction and hyperglycemia could then induce B1 expression through NF-κB [27,28].

It is important to mention, that during evaluation of the bradykinin receptors expression in both groups, placental tissue slides were matched with the vascular density. It is very likely, that increased mean vascular density in diabetic placenta will affect the results, potentiating the differences between examined groups. Angiogenic properties of histamine and bradykinin as well as of the another mast cell derived mediators influence on local vascular density in diabetes [29,30]. The growth-promoting activities of bradykinin are potentiated by insulin via calcium channel. Thus, hyperinsulimemic conditions due to insulin resistance may potentiate these actions of bradykinin [31]. Increased kininogen levels observed in plasma of diabetic patients can be corrected by administration of insulin [32]. The role of insulinotherapy should be taken into account. Another line of investigation suggests that kinins exert protective effects in diabetes models in rodents as inhibitors of kininases improved the sensitivity to insulin [33].

It should be noticed, that we studied histamine concentration and bradykinin receptors expression in well-controlled White’s class C placentae, that means: without significant vascular changes. Regulation of the blood flow in the human placenta by changes of the vascular resistance is made unique by the lack of sympathetic innervation in placental vessels [34]. For that reason, humoral factors (e.g. histamine, bradykinin) are more important than elsewhere. Both, histamine and bradykinin are vasoconstrictors acting within the placental vasculature. Increased local vascular resistance due to histamine excess in diabetic placenta seems to be of greater importance, because is accompanied by lowered activity of histamine degrading enzyme, monoamine oxidase (EC 1.4.3.4.) [35] and increased activity of HDC. Unchanged placental B2 receptors expression and increased activity of the placental proteases (EC 3.4.) in diabetes may attenuate vasoconstrictor activity of bradykinin, even if bradykinin is present in higher concentrations [22].

In conclusion, increased amounts of histamine in placental tissue in diabetes producing pro-inflammatory conditions may change vascular properties to the some degree by influence on bradykinin receptors expression and vice versa (i.e. bradykinin may affect action of histamine). Pro-inflammatory reactions mediated via B1 should be expected rather, than changed vasmotor reactivity related to B2. Angiogenic properties of histamine and kinins should also be considered.

References: