New Placental Vasoactive Factors and Gestational Diseases

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During normal pregnancy, important physiological adaptations occur in the mother which assure an adequate blood supply to the fetus. Vascular resistance, mean arterial pressure, and sensitivity to endogenous constrictors are reduced, in addition to increased cardiac output, heart rate, and blood volume. Alteration of these haemodynamic adaptations to pregnancy is associated with conditions critical to fetal well-being such as pre-eclampsia (PE) and intrauterine-growth retardation (IUGR), characterized by endothelial dysfunction. The human placenta is a multifunctional organ providing oxygen, water homeostasis, nutrition and endocrine messages for the fetus until delivery. Impaired placental perfusion is associated with reduced transfer of oxygen and nutrients from the mother to the fetus. Consequently, fetal growth and oxygenation are reduced resulting in intrauterine growth retardation (IUGR) and fetal hypoxemic hypoxia. In humans, increased placental resistance may be the result of different factors: reduction in the number of placental terminal capillaries and small muscular arteries in the tertiary stem villi, increased vasoconstriction at villous level because of local release of vasoactive substances, e.g. thromboxane or endothelin, or decrease of vasorelaxant agents. These alterations are triggered by ischemia of the intervillous space due to reduced utero-placental perfusion.

The mechanisms involved in the control of fetal-placental vascular tone remain to be clearly characterized as well as the physiological response of the fetus to adverse circumstances during pregnancy. Since placenta lacks autonomic innervation, vascular tone at this level is regulated by the action of endothelial derived factors; thus it is well defined the central role of the vasoactive substances produced by the placenta and by the fetus himself in the regulation of placental and fetal circulation. Among these substances a key role may be played by some newly discovered agents such as adrenomedullin (AM), nitric oxide (NO) and endothelin (ET-1) in physiological and pathophysiological conditions. In this light, we have investigated the potential role of these vasoactive factors in some gestational diseases such as PE, IUGR and gestational diabetes, conditions in which there are evidences of endothelial dysfunction.

We measured AM, NO and ET-1 concentrations in maternal, fetal and placental compartment by means of specific RIA, moreover, we investigated their prevalence and distribution on placental tissues and cord by means of immunohistochemistry.

Endothelin

Endothelin (ET) is a 21-amino-acid peptide made by from pre-pro-ET (200 amino-acids) and pro-ET (38 amino-acids, called "big-ET") by a cleavage performed by an ET converting enzyme (ECE) (1). The family of endothelin peptides (ET) consists of three distinct isoforms: ET-1 (the original porcine and human ET), ET-2 (with two amino-acid substitutions), and ET-3 (with six amino-acids substitutions) coded for by three separate genes in the human, rat, and porcine genome (2). ET was first cloned from a human placental cDNA library, it has been demonstrated that the human placenta, and uterus, have specific high-affinity receptors for ET and that the peptide increases vascular resistance in the fetoplacental vasculature. ET-induced pressor responses are of long duration and are associated with an involvement of other vasoactive substances. The data are compatible with a role of ET as an endogenous modulator of the fetoplacental circulation in humans. ET-1 has been localized in placenta and fetal membranes (3), and mRNA for ET-1 has been found in endothelial cells of placental villi and in avascular amnion of fetal membranes (4). Accordingly to other studies (5-7) we fo-

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und that in PE and IUGR pregnancies ET-1 concentrations in placental compartment were higher than in normotensive pregnancies (8). Among complicated pregnancies, amniotic fluid levels were significantly higher in patients with PE associated to IUGR than in PE alone women. We demonstrated increased concentrations of ET-1 also in pregnancies complicated by gestational diabetes (9). All these conditions are characterized by endothelial damage, and higher levels correlated with a more severe impairment of placental vasculature, with reduction of blood flow to the fetus. ET-1 may play a significant role in determining a potent vasoconstriction of placental vessels, and activating other factors (e.g prostaglandins, thromboxane A2, PAF) responsible for the hemodynamic alterations. ET-1 production by placental tissues may precede and thus contribute to the development of increased placental vascular resistances. Alternatively, the elevated ET-1 levels may be the results of endothelial damage associated with increased placental resistances.

Nitric oxide

Nitric oxide (NO) is a potent vasodilator released from L-arginine by endothelial cells that plays a key role in the control of vascular tone in several districts. There is evidence that NO is released by the feto-placental tissues. NO synthase is present in the human fetal-placental tissues and endothelial NO synthase mRNA has been detected in the placental villi (10). Furthermore several groups have reported that NO donors such as glyceryl trinitrite or L-arginine relax human umbilical artery and NO decreases perfusion pressure of the human fetoplacental circulation (11,12). These studies indicate that NO produced by fetoplacental tissues may contribute to the regulation of blood flow from and to the fetus. In vitro studies have shown the inhibition of NO release in placental circulation induces an increase in arterial pressure, amplifying vascular response to vasoconstrictive autacoids. Several studies have addressed NO production in PE, although resulting are conflicting. Maternal serum, urine and cord blood NO metabolites concentrations are reported increased, reduced, or unchanged in preeclamptic women (13,15). Recently a calcium-dependent nitric oxide synthase (NOS), corresponding to the endothelial isoform of the enzyme (eNOS) has been localized on syncytiotrophoblast and endothelial cells of human placental villi and its activity assayed in placental extracts (16). In a recent study we found that amniotic fluid NO concentration was significantly higher in PE women. Moreover, we found that in placental tissues collected from PE the intensity of the staining and the percentage of endothelial cells stained for the eNOS were higher than in the control group. Our immunohistochemical data confirm the study of Ghabour et al. (18) on placental NOS expression in PE, suggesting that, in the endothelial cells of the villi, eNOS expression is enhanced in PE patients and can help to maintain the placental vasculature maximally dilated. The higher concentrations of amniotic fluid nitrate in PE patients may result from the stimulation of placental NOS activity. The significance of this increased activity could be compensatory for the increased synthesis and release of others vasoactive substances (TxA2, ET-1, etc.) from the injured endothelium acting as vasoconstrictors on placental vasculature. This hypothesis is supported by the finding of increased NO levels in amniotic fluid of IUGR pregnancies with normal feto-placental haemodynamics, whereas NO metabolites concentrations are significantly reduced in IUGR with abnormal uterine artery resistance index. Furthermore, nitrite levels in amniotic fluid correlated inversely with uterine resistance index and the umbilical artery PI/fetal middle cerebral artery PI ratio, an index of fetal redistribution of circulation which is characterised by peripheral vascular contraction and cerebral vasodilatation (19). These data suggest that in IUGR pregnancies a compensatory increase in the synthesis of NO within the placenta may occur in an attempt to maintain an adequate blood flow through the placenta. When NO production decreases, resistance in utero-placental and fetal-placental circulation markedly increases, compromising fetal haemodynamics. This offers the rationale for the use of NO-donors in IUGR pregnancies complicated by IUGR with impaired utero-placental circulation.

Adrenomedullin

Adrenomedullin (AM) is a vasoactive peptide first isolated in pheochromocytoma produced by endothelial and vascular smooth muscle cells. During pregnancy AM concentrations are significantly increased and high levels are present also in fetal plasma and amniotic fluid (20). The placenta is an important site of production for this peptide during pregnancy, and, because of its vasodilator and hypotensive effects it has been suggested that AM could participate in the physiological modifications of maternal haemodynamic in pregnancy (21). Moreover acting in an autocrine, paracrine manner, it may have a role also in the regulation of utero-pla-

centa and fetal circulation. The placental vessels are relaxed by AM in a dose-dependent manner and AM is expressed in the fetoplacental and umbilical vascular endothelium where basal production of AM contributes to low fetoplacental vascular resistances. AM induces relaxation trough an increase in cAMP with the reduction of intracellular Ca2+, but also indirectly stimulating NO release and inhibiting ET-1 secretion by endothelial and vascular smooth muscle cells (22). Infusion of NO-LA, which acts inhibiting NO production causes an attenuation of AM induced vasodilatation. NO is an important mediator of placental vascular tone, thus regulating blood support to the fetus (23).

Maternal circulating AM has been reported either increased, decreased or unchanged (24-26) , whereas in umbilical plasma and amniotic fluid we found that its concentrations were higher than in normotensive pregnancies (27). Conflicting results have been reported also in the expression of AM in feto-placental tissues in PE. Ir-AM in placentas of PE women was found decreased or unchanged, and AM mRNA expression has been shown to be either decreased or unchanged in the placenta and uterine muscle, decreased in fetal membranes and increased in umbilical artery (28). Recently we found that in IUGR fetuses AM concentration was higher than in normal pregnancy and it correlated with cerebral and peripheral blood flow (29). Of particular significance seems to be the role of AM in the fetal response to hypoxia as demonstrated by the relationship between circulating AM and vasodilatation of fetal cerebral vessels. In another condition associated with endothelial damage such as gestational diabetes we found that AM concentrations in amniotic fluid were higher in pregnant women with diabetes, both preexisting or manifested during pregnancy, than in euglycemic controls whereas no differences were found in maternal circulation (30). This finding suggests an enhanced synthesis or secretion of the peptide by the placental structures that may play a regulatory function, preventing excessive vasoconstriction and inhibiting excessive platelet aggregation. This hypothesis is supported by the finding of a negative correlation between amniotic fluid AM levels and arterial blood pressure in diabetic pregnant women.

Summary

In summary we found that all these newly discovered vasoactive factors are produced by the placental tissues in large amount and are secreted in the fetal compartment where they participate in the regulation of feto-placental circulation. In pregnancy complicated by PE, IUGR and gestational diabetes, conditions associated with impairment of utero-placental and fetal hemodynamics, ET-1, NO and AM secretion is significantly affected.

These findings suggest a potential future use of these vasoactive factors in the clinical management of these pregnancy complications.

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