Effects of selected endothelium-dependent vasodilators on fetoplacental vasculature: physiological implications

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Amarnani, Saral, Belinda Sangrat, and Gautam Chaudhuri. Effects of selected endothelium-dependent vasodilators on fetoplacental vasculature: physiological implications. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H842–H847, 1999.—The endothelium-dependent vasodilators ACh, histamine, and bradykinin were studied in the isolated, perfused human placental cotyledon. Histamine caused a decrease in perfusion pressure that was attenuated by d-methyldopa. Bradykinin, at lower concentrations (10⁻²⁰ to 10⁻¹⁴ M), produced a concentration-dependent decrease in perfusion pressure, whereas at higher concentrations it produced an increase in perfusion pressure. ACh was without any effect. The decrease in perfusion pressure observed with bradykinin was potentiated by captopril and was significantly attenuated in the presence of HOE-140, the B₂-receptor antagonist, or by pretreatment with an inhibitor of nitric oxide synthase, but not by an inhibitor of cyclooxygenase. The decrease in perfusion pressure observed with bradykinin was potentiated by ANG I but not by ANG II. It is concluded that endothelium-dependent vasodilation can be demonstrated with histamine and bradykinin in the fetoplacental vessels, and at least for bradykinin, this is partly mediated by release of nitric oxide. The potentiation of the bradykinin response in the presence of ANG I may serve to buffer the vasoconstriction produced by ANG II in the fetoplacental circulation.

bradykinin; human cotyledon perfusion; nitric oxide

THE FETOPLACENTAL CIRCULATION is very important for supplying oxygen and nutrients to the fetus to allow for normal fetal growth and viability. These vessels lack innervation (20), and regulation of vascular tone in the fetal extracorporeal circulation must therefore depend on circulating vasoactive substances that may act locally in the vasculature to modulate vascular tone. Any impairment in the circulation could increase the possibilities of growth retardation of the fetus in utero, fetal distress, and intrauterine fetal death (2). We (3, 4) and others (22) have previously demonstrated that histamine, bradykinin, and other endothelium-dependent vasodilators release nitric oxide (NO) from umbilical artery and vein. It has also been demonstrated that NO regulates vascular tone in the fetoplacental vessels (12). However, the actions of endothelium-dependent vasodilators in eliciting vasodilation of fetoplacental vessels have been controversial. Although histamine has been demonstrated to release endothelium-derived relaxing factor (EDRF), now identified as NO (9, 16), from human umbilical vessels and umbilical endothelial cells in culture (22), endothelium-dependent relaxation of isolated segments of human umbilical artery has been difficult to demonstrate (11). Similarly, the actions of bradykinin in the fetoplacental vessels have been conflicting. Some investigators have reported that bradykinin infusion increased perfusion pressure in the placenta (23), whereas others observed no change (5, 17).

Both histamine (19) and bradykinin (10) are present in the fetal circulation. We therefore decided to reassess the role of these endothelium-dependent vasodilators in causing vasodilation of the fetoplacental vessels. Because both histamine and bradykinin have vasodilator and vasoconstrictor actions, we decided to utilize much lower concentrations of these two substances than previously utilized by other investigators to assess whether endothelium-dependent vasodilation can be demonstrated with very low concentrations. We also decided to assess the potential mechanism(s) by which bradykinin modulates tone in these vessels and their physiological implications.

MATERIALS AND METHODS

Isolated Human Placental Cotyledon Perfusion

Human placentas were collected at the University of California, Los Angeles, delivery room immediately after normal vaginal delivery or elective cesarean section and were immediately transported to the perfusion laboratory. The isolated human placental cotyledons were dually perfused using the technique described by Glance et al. (6). A suitable third- or fourth-order chorionic artery and corresponding vein of an intact cotyledon were cannulated at a point immediately before passage of the vessels through the chorionic plate. The maternal intervillous space was cannulated with two butterfly needles inserted through remnants of the spiral arteries in the basalis plate. The effluent from the intervillous space was collected by gravity into a Plexiglas cone over which the cotyledon was placed, maternal surface facing downward. Perfusion medium in most cases was tissue culture medium 199 containing 5% polyvinylpyrrolidone-40 and 0.1% BSA as oncotic agents, with 20 IU/ml heparin sodium and 48 µg/ml gentamicin added. In some experiments, when NO synthase inhibitors were used, arginine-free medium was utilized. The pH of the medium was 7.4 and was gassed with 95% O₂-5% CO₂ at 37°C. Flow rates were 4 ml/min for the fetal side and 10 ml/min for the maternal side except where stated. Lateral pressure was measured on fetal and maternal inflow lines adjacent to the point of cannulation. Data on pressure, inflow, and outflow were constantly monitored. The PO₂, when sampled ranged from 380 to 500 mmHg. The pH was maintained at 7.4. Because flow was kept constant, changes in perfusion pressure were reflective of changes in tone of the vessels.

Active tone was induced in the fetal side of the placental vasculature by adding the thromboxane mimetic agent
U-46619 in a final concentration of 1–5 × 10⁻⁸ M. This led to an increase in fetoplacental perfusion pressure ranging from 100 to 130 mmHg at a flow rate of 4 ml/min. The endothelium-dependent and endothelium-independent vasodilators or other agents studied were added to the perfusion medium directly into the fetal inflow line via an injection valve, over a wide range of concentrations as indicated in the respective protocols. In some instances, when antagonists to the endothelium-dependent vasodilators were utilized as indicated, they were added to the perfusion medium and allowed to constantly perfuse the vascular bed of the cotyledon for 30 min before the response to the endothelium-dependent vasodilators was retested. In all instances, at the end of the experiment, the responsiveness of the vascular bed was tested by perfusion of 10⁻⁶ M glyceryl trinitrate (GTN). This concentration of GTN induced maximal vasodilation and decrease in perfusion pressure in our model system. The results of the studies were discarded if the vascular bed failed to respond to GTN at the end of the experimental protocol.

Chemicals and Solutions

ACh, bradykinin, histamine, GTN, captopril, ANG I, ANG II, N-nitro-L-arginine methyl ester (L-NAME), and L-arginine (L-Arg) were obtained from Sigma (St. Louis, MO). Tissue-culture medium and arginine-free medium were obtained from GIBCO and Specialty Media, respectively. HOE-140 (selective B₂ bradykinin receptor antagonist) was obtained from RBI (Natick, MA).

Experimental Protocols

Study I: Effects of different concentrations of ACh, histamine, and bradykinin on the placental perfusion pressure and vascular resistance. After stabilization of the perfusion pressure after induction of active tone in the fetal side of the placental vasculature, the test substances in appropriate concentrations were injected into the fetal inflow line over a period of 5 min. At least 15 min were allowed to elapse between each infusion of drug or until a steady baseline was again achieved. The test substances studied were ACh (10⁻⁶ to 10⁻⁴ M), histamine (10⁻⁸ to 10⁻⁶ M), and bradykinin (10⁻¹⁰ to 10⁻⁸ M). The effects of histamine were assessed in the absence and presence of cimetidine (10⁻⁵ M). The effects of bradykinin were assessed in absence or presence of 0.1 µM HOE-140 (the B₂-receptor antagonist).

Study II: Effect of inhibition of cyclooxygenase and endothelial NO synthase on the responses to bradykinin. For these studies, L-Arg-free perfusion medium was used. The effects of bradykinin (10⁻⁶ to 10⁻¹⁴ M) were initially ascertained followed by a 30-min infusion of indomethacin (10⁻⁵ M) to inhibit cyclooxygenase. This concentration of indomethacin was selected because it is known to completely inhibit cyclooxygenase (4). After this, the effects of different concentrations of bradykinin in the presence of indomethacin were reassessed. After this, L-NAME (10⁻⁴ M) was added to the perfusion medium, and the concentration of U-46619 was adjusted to ensure that the perfusion pressure was similar to that obtained before the addition of L-NAME. The effects of bradykinin were again retested.

Study III: Effects of bradykinin in the presence and absence of captopril, ANG I, and ANG II. In this series of experiments, we initially assessed whether the effects of bradykinin were modified in the presence of captopril, a converting enzyme inhibitor. We then assessed whether a similar modification of bradykinin action occurred in the presence of ANG I which gets converted by the converting enzyme to ANG II.

Initially, the responses to bradykinin (10⁻¹⁸ to 10⁻¹⁴ M) were elucidated in the absence of captopril. After this, captopril (10⁻⁷ M) was added to the perfusion medium and was perfused for at least 30 min before the responses to bradykinin in the presence of captopril were reassessed. This concentration of captopril was selected because it has been previously shown to significantly inhibit the converting enzyme in the fetoplacental vasculature (1).

In separate experiments, the same protocol was utilized, but instead of captopril, ANG I (3 × 10⁻⁸ M) or ANG II (2 × 10⁻⁸) was used. The concentrations of ANG I and ANG II perfused were selected on the basis of their reported concentrations in the feto-placental circulation (14, 15).

Data Analysis

Decrease in mean perfusion pressure was measured as the percent decrease in mean pressure below the stable perfusion pressure elicited by inducing active tone in the fetoplacental vessels by U-46619. Increase in mean pressure above the stable perfusion pressure was measured as the percentage of increase in perfusion pressure elicited by inducing active tone with U-46619. Values are expressed as means ± SE. Comparisons of means were made by using Student’s t-test or ANOVA with repeated measurements where appropriate. If a significant F-value was found, a Bonferroni comparison between means was performed. Differences were considered to be significant at P < 0.05. Four to seven placenta were used for each experimental protocol.

RESULTS

Study I: Effects of Different Concentrations of ACh, Histamine, and Bradykinin on the Placental Perfusion Pressure and Vascular Resistance

ACh (10⁻⁶ to 10⁻⁴ M) was without any effect on the fetoplacental vasculature (data not shown). Histamine (10⁻⁶ to 10⁻⁴ M) led to a concentration-related relaxant effect (Fig. 1), and this was partially abolished by cimetidine (Fig. 2). Bradykinin caused a biphasic response. Usually at low concentrations (10⁻¹⁰ to 10⁻¹⁶ M), there was a concentration-dependent relaxation. This was followed by a diminution in the relaxant effect.

Fig. 1. Tracing showing effects of different concentrations of histamine (Hist) on perfusion pressure in isolated, perfused human placental cotyledon. Histamine administration to fetal side of placental vasculature led to a concentration-dependent decrease in perfusion pressure.
followed by contraction (Fig. 3). However, there was considerable interplacental variation in the response of the fetoplacental vessels to bradykinin. The concentration of bradykinin when a biphasic response was first observed also varied between different placentas. The effects of low concentrations of bradykinin (10^{-20} to 10^{-16} M) were significantly attenuated in the presence of HOE-140, the B2 receptor antagonist (Fig. 4).

Study II: Effect of Inhibition of Cyclooxygenase and Endothelial NO Synthase on the Responses to Bradykinin

Bradykinin at 10^{-16} M produced a vasodilator response followed by a biphasic response at 10^{-14} M. There was initially a decrease in perfusion pressure with 10^{-14} M bradykinin, and this was followed by an increase in perfusion pressure. After pretreatment with indomethacin, the decrease in perfusion pressure at the two concentrations was unaltered. However, the increase in perfusion pressure observed with 10^{-14} M bradykinin was significantly attenuated (Fig. 5). After addition of L-NAME, there was significant attenuation of the decrease in perfusion pressure observed with both concentrations of bradykinin when compared with the values observed in the absence of L-NAME (Fig. 5).

Study III: Effects of Bradykinin in the Presence and Absence of Captopril, ANG I, and ANG II

The vasodilator action of bradykinin (10^{-16} to 10^{-14} M) was significantly potentiated in the presence of captopril (Figs. 6 and 7). Similarly, the effects of lower concentrations of bradykinin (10^{-20} to 10^{-16} M) were potentiated in the presence of ANG I but not in the presence of ANG II (Fig. 8).

DISCUSSION

The objectives of the present study were to reassess the effects of some selective endothelium-dependent vasodilators on the fetoplacental vessels and their potential physiological role with special relevance to bradykinin. We utilized the dually perfused, isolated human placental cotyledon for this study because this would allow the endothelium-dependent vasodilators to initially come in contact with the endothelium when these substances are added to the perfusate. This would be more physiological than utilizing isolated strips of vessels set up in organ baths where the vasodilators may have actions on both the endothelium, releasing an endothelium-derived vasodilator, and a direct contractile action on the vascular smooth muscle, thereby producing opposing actions that may not have physiological relevance.

Our observations that ACh did not produce any decrease in perfusion pressure, reflecting an absence of vasodilatory action in the fetoplacental vessels, is similar to that reported by other investigators (25) and most likely reflects an absence of the muscarinic receptors of ACh on endothelial cells. In this regard, these vessels appear to be different from adult vessels where the ACh...
receptors are invariably present on endothelial cells and ACh produces relaxation of both conduit and resistance vessels. The physiological implications for the absence of ACh receptors on fetoplacental endothelial cells is not known. Histamine, on the other hand, produced a concentration-related decrease in perfusion pressure in the isolated, perfused human placental cotyledon preparation similar to that reported by others (13), indicating that histamine is able to relax fetoplacental vessels. This action of histamine is most likely due to its ability to release EDRF, now known as NO (9, 16), as has been previously demonstrated by us and others (3, 22). Our observations indicate that this vasodilation produced by histamine is most likely mediated by the H2 receptors, because cimetidine, an H2-receptor antagonist, was able to significantly attenuate this action of histamine. One group of investigators demonstrated the ability of histamine to release EDRF from perfused human umbilical artery, vein, and umbilical vascular endothelial cells in culture but failed to observe any relaxation of umbilical vascular preparations by histamine when these preparations were mounted for isometric tension recording precontracted with 5-hydroxytryptamine. On this basis, these authors suggested that the umbilical arteries may have poor relaxant response to cGMP-mediated vascular relaxation (22). This does not appear to be the case, because we (3, 4) and others (12) have previously demonstrated that fetoplacental vessels do relax by cGMP-mediated mechanisms and that NO is more potent than prostacyclin in relaxing fetoplacental vessels. The most likely explanation may be that in our experiments histamine added to the perfusate initially came in contact with the endothelial cells, leading to release of NO and thereby producing vascular relaxation. On the other hand, when isolated tissues in organ baths were utilized, the endothelial cells and vascular smooth muscles were simultaneously exposed to histamine, and the histamine, acting via H1 receptors, may have produced constriction of the vessels that may have counteracted any actions of NO released by endothelial cells. Other investigators have also reported a decrease in perfusion pressure with histamine using the dually perfused, isolated human placental cotyledon (13).

The actions of bradykinin on the fetoplacental vessels are controversial. Some investigators have reported that bradykinin infusion increased perfusion pressure in the placenta (23), whereas others (5, 17) observed no change. On this basis, we elected to assess the effects of very low concentrations of bradykinin on the fetoplacental vessels. Our observations are interesting that bradykinin at very low concentrations decreases perfusion pressure, indicating a vasorelaxant effect, and that at higher concentrations increases perfusion pressure, indicating a vasoconstrictive action. Bradykinin and related kinins act via two receptors designated as B1 and B2 receptors (18). Bradykinin has a high affinity for binding to the B2 receptors and a low affinity for binding to B1 receptors (21). At low concentrations, when perfused intraluminally, bradykinin comes in contact with the endothelial cells and leads to a release of NO. This action of bradykinin was mediated by the B2 receptors because the actions of bradykinin were significantly attenuated when bradykinin was perfused in the presence of the B2-receptor antagonist HOE-140. This vasodilator action of bradykinin was mediated by release of NO and not by the
vasodilator prostanoids because the vasodilator action of bradykinin was significantly attenuated only in the presence of L-NAME but was not affected by indomethacin, an inhibitor of cyclooxygenase. As higher concentrations of bradykinin were perfused, a biphasic response was observed over certain concentration ranges followed by a vasoconstrictor response. The concentrations at which this biphasic response was observed varied slightly from one placenta to the other and most likely represent variation in response to bradykinin in different placentas. The abolition of a slight vasoconstrictor response to bradykinin observed after pretreatment with indomethacin was most likely due to inhibition of thromboxane $A_2$ release since this was abolished by indomethacin. The vasoconstrictor response observed with higher concentrations of bradykinin may be due to the bradykinin being able to diffuse through the endothelial cell barrier and coming in contact with the vascular smooth muscle, leading to vascular smooth muscle contraction and thereby negating the vasorelaxant effects of any NO released by bradykinin from the endothelial cells. Our studies therefore indicate the importance of using very low concentrations of endothelium-dependent vasodilators and perfusing these substances intraluminally to demonstrate endothelium-dependent vasodilation in fetoplacental vessels. These factors may be the potential explanation for the contradictory observations by other investigators where much higher concentrations of bradykinin have been utilized in studies with perfused isolated human placental cotyledons or in studies utilizing isolated vascular strips of fetoplacental vessels in organ baths.

Our observation that bradykinin at low concentrations leads to vasodilation of fetoplacental vessels may have important physiological implications. Concentrations of free bradykinin are higher in umbilical venous and arterial cord blood than in maternal venous blood (10). Bradykinin causes pulmonary vasodilation in the fetus, and it has been shown that bradykinin is released following oxygenation of the fetal lungs and that it is responsible at least in part for the normal pulmonary vasodilation seen in the transition from fetal to extraterine life (8). It is possible that bradykinin is also responsible for maintaining the fetoplacental vessels in a vasodilated state. These vessels are not innervated (20); therefore, endogenous circulating vasodilators must play an important role in modulating tone in these vessels. Angiotensin-converting enzyme in the fetoplacental vascular bed plays a key role in the regulation of fluid balance in the fetus by converting ANG I to ANG II. This enzyme also inactivates bradykinin (5). Our observation that the vasodilator effects of low concentrations of bradykinin were potentiated by captopril, an inhibitor of the converting enzyme, indicates that bradykinin is degraded by this enzyme in the fetoplacental circulation. Other investigators who have utilized higher concentrations of bradykinin and observed a constrictor response have reported that captopril potentiated the vasoconstrictor action of bradykinin in the human fetal placental circulation (5). These findings support our observation that converting enzyme is present in the fetal placental vascular bed.

Our observation that ANG I at concentrations present in the fetal circulation but not ANG II (14, 15) potentiated the vasodilator effect of bradykinin in the fetoplacental vessels may also have important physiological implications. It has been demonstrated (14, 15) that the level of plasma ANG II is higher in cord blood than in the maternal circulation after vaginal delivery. The fetal pulmonary circulation has a high vascular resistance and low blood flow because of the cardiopulmonary bypasses through the ductus arteriosus and foramen ovale. Thus it is not likely that the fetal lung is responsible for the conversion of ANG I to ANG II. The placenta is one of the likely sites for this conversion (24). However, the ANG II produced in the fetoplacental circulation could lead to vasoconstriction and increase in perfusion pressure as has been demonstrated in the isolated perfused human placental cotyledon (7). However, if bradykinin competes with ANG I for the converting enzyme leading to decreased degradation of bradykinin and thereby potentiating the vasodilatory action.
of bradykinin, this could then serve as a buffering mechanism to counteract the simultaneous vasoconstriction produced by ANG II formed from ANG I by the converting enzyme. Such a local buffering mechanism must exist to counteract uncontrolled vasoconstriction in these noninnervated fetoplacental vessels by the ANG II produced in order for the fetus to survive.

In conclusion, our results indicate that selective endothelium-dependent vasodilators can cause endothelium-dependent vasodilation in fetoplacental vessels. The action of these vasodilators, specifically bradykinin, may have important physiological relevance in maintaining vasodilation in the fetoplacental circulation.

This work was supported in part by National Institute of Child Health and Human Development Grants HD-31467 and HD-37465. Address for reprint requests and other correspondence: G. Chaudhuri, Dept. of Obstetrics and Gynecology and Molecular and Medical Pharmacology, UCLA School of Medicine, Los Angeles, CA 90095-1740 (E-mail: gchaudhu@obgyn.medsch.ucla.edu).

Received 11 December 1998; accepted in final form 23 March 1999.

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