Involvement of calcitonin gene-related peptide in control of human fetoplacental vascular tone

Yuan-Lin Dong, Sujatha Vegiraju, Madhu Chauhan, Pandu R. R. Gangula, Gary D. V. Hankins, Linda Goodrum, and Chandra Yallampalli

Department of Obstetrics and Gynecology, The University of Texas Medical Branch, Galveston, Texas 77555-1062

Submitted 19 February 2003; accepted in final form 22 August 2003

Dong, Yuan-Lin, Sujatha Vegiraju, Madhu Chauhan, Pandu R. R. Gangula, Gary D. V. Hankins, Linda Goodrum, and Chandra Yallampalli. Involvement of calcitonin gene-related peptide in control of human fetoplacental vascular tone. Am J Physiol Heart Circ Physiol 286: H230–H239, 2004; 10.1152/ajpheart.00140.2003.—Calcitonin gene-related peptide (CGRP), one of the most potent endogenous vasodilators known, has been implicated in vascular adaptations and placental functions during pregnancy. The present study was designed to examine the expression of CGRP receptor components, the calcitonin receptor-like receptor (CRLR) and receptor activity-modifying protein 1 (RAMP1), in the human placenta and the vasoreactivity of CGRP in the fetoplacental circulation. Immunofluorescent staining of the human placenta in term labor using polyclonal anti-CRLR and RAMP1 antibodies revealed that labeling specifically concentrated in the vasculature and the underlying smooth muscle cells in the umbilical artery/vein, chorionic artery/vein, and stem villous vessels as well as in the trophoblast layer of the placental villi. In vitro isotropic force measurement showed that CGRP dose dependently relaxes the umbilical artery/vein, chorionic artery/vein, and stem villous vessels. Furthermore, CGRP-induced relaxation of placent al vessels are inhibited by a CGRP receptor antagonist (CGRP8–37), ATP-sensitive potassium (KATP) channel blocker (glybenclamide), and cAMP-dependent protein kinase A inhibitor (Rp-cAMPS) and partially inhibited by a nitric oxide inhibitor (N“-nitro-L-arginine methyl ester). We propose that CGRP may play a role in the control of human fetoplacental vascular tone, and the vascular dilations in response to CGRP may involve activation of KATP channels, cAMP, and a nitric oxide pathway.

pregnancy; cell signaling; vasoactivity; trophoblast; fetus

THE PLACENTA IS A CRITICAL ORGAN for fetal growth and development and is responsible for the transfer of nutrients, ions, and lipids from the mother to fetus. Regulation of placental blood flow in both maternal and fetal compartments affects hormonal production and the transport of oxygen and nutrients, which ultimately determines fetal growth and well being. Unlike other vascular systems, the fetoplacental unit lacks innervation and therefore depends on humoral substances for the control of vascular tone (8).

Calcitonin gene-related peptide (CGRP) is one of the most potent endogenous vasodilators known (12). This 37-amino acid peptide is produced by alternative processing of mRNA from the calcitonin gene (46). CGRP is primarily synthesized in the sensory neurons of dorsal root ganglia (DRG), which extend axons centrally to the spinal cord and peripherally to various organs including blood vessels (7, 30), and is present in the bloodstream (2). In the pregnant woman, the serum levels of CGRP are significantly increased in both maternal and fetal circulations (42). The magnitude of increases in fetal serum CGRP is related to the fetal weight and gestational age (34), indicating the possible involvement of this neuropeptide in fetal growth and development.

CGRP exerts its biological action by interacting with its receptors. It is well recognized that the calcitonin receptor-like receptor (CRLR) functions as either a CGRP receptor or an adrenomedullin receptor depending on the expression of the type of receptor activity-modifying proteins (RAMPs) (32). RAMP1 presents the CRLR at the cell surface as a CGRP receptor, whereas RAMP2 and RAMP3 transport CRLR as an adrenomedullin receptor. Recently reported data suggest the presence of two types of CGRP receptors, CGRP receptor type A and B (9, 45). The CGRP-A receptor consists of two components, CRLR and RAMP1, and the CGRP-B receptor is a distinct receptor that is not related to CRLR (45). At the present time, however, the expression of CRLR and RAMP1 in the human placenta and their cellular localization remain unknown.

Recently, we (18) have demonstrated in a rat model that subcutaneous infusion of CGRP8–37, an antagonist of CGRP, caused significant reduction in pup weight with an increase in fetal mortality rate, and these effects were dose dependent, implying that endogenous CGRP may play a role in fetal development. In the human, it has been reported that CGRP causes relaxation of chorionic plate vasculature (17) and reduces placental vascular resistance in perfused placental cotyledons (29), suggesting a beneficial role for CGRP in uteroplacental vascular relaxation. However, several issues remain unclear: 1) whether CGRP differentially relaxes the umbilical artery and vein, chorionic artery and vein, and stem villous vessels in vitro; 2) whether the effects of CGRP on fetoplacental vessels are mediated through the CGRP receptors; and 3) if the vascular response to CGRP involves activation of ATP-sensitive potassium (KATP) channels, cAMP stimulation, or the nitric oxide pathway. Therefore, present study was designed to examine the expression of CGRP-A receptor components CRLR and RAMP1, the effects of CGRP on vascular tone of various fetoplacental vessels, and the postreceptor signaling pathway of CGRP in human fetoplacental tissues.

METHODS

Human subjects. The study population consisted of normotensive pregnant women who were admitted to the Department of Obstetrics and Gynecology of the University of Texas Medical branch at...
Table 1. Demographic data of the patients

| Maternal age, yr   | 32.8 ± 1.7
| Gestational age, wk | 38.5 ± 0.5
| Birth weight, g    | 3,708 ± 246
| Gravidity          | 4.5 ± 0.9
| Parity             | 2.3 ± 0.8

Values are means ± SE; n = 6 patients. Of the 6 placentas obtained from the patients, 4 were from cesarean sections.
2.5 mM calcium chloride, 25 mM sodium bicarbonate, 11.1 mM dextrose, and 0.034 mM sodium ethylenediaminetetraacetic acid) and processed as described by Belfort et al. (4). Briefly, the vessels were cut into 4-mm rings, mounted onto stainless steel wire stirrups (200 μm), and placed in 5-ml organ baths containing Krebs solution maintained at 37°C. A gas mixture of 5% CO₂-21% O₂-74% N₂ was constantly bubbled through the organ bath solution. The isometric force generated by the vessels was monitored with isometric transducers (Harvard Apparatus; South Natick, MA) and analyzed with a DATAQ system (DATAQ Instruments; Akron, OH). The passive tension was gradually increased to the optimal level of 2 g during an equilibration period of 2 h. Each vessel ring was contracted repeatedly with potassium chloride (60 mM) until a stable contraction was obtained. After the potassium chloride was washed out, the ED₇₀ of serotonin (5-HT) was determined, and 5-HT at ED₇₀ was then used as the precontracted dose for the CGRP dose response. The main stem villous vessels of 2 mm in length were inserted with tungsten wires through the lumen and mounted onto two Teflon blocks of a wire myograph (Kent Scientific; Litchfield, CT). The chambers containing Krebs solution were bubbled with the gas mixture described above. The vessels in the chambers were stretched 200–225 μm for 15 min and depolarized with potassium chloride (60 mM), followed by the assessment of ED₇₀ for the thromboxane A₂ mimetic U-46619. Varying concentrations of CGRP (10⁻¹⁰–10⁻⁶ M) were applied to the chamber in a cumulative manner. The relaxation responses to CGRP were calculated as a percentage of the 5-HT-induced initial tension of the vessel. To examine the cell signaling pathway of CGRP, we incubated the vascular segments for 30 min in fresh Krebs solution with either CGRP₈₋₃₇ (10⁻³ M, a CGRP receptor antagonist), glybenclamide (10⁻⁵ M, a K⁺ ATP channel blocker), Rp-cAMPS (10⁻⁵ M, a cAMP-dependent protein kinase inhibitor), or L-nitro-arginine methyl ester (l-NAME; 10⁻⁴ M, a nitric oxide synthase inhibitor). In these experiments, the logEC₅₀, the CGRP concentration at which the initial tension was reduced by 50%, was also calculated using a nonlinear regression curve system (Prism, GraphPad Software; San Diego, CA).

Fig. 3. Immunofluorescent localization of CRLR (A and D) and RAMP₁ (B and E) in the human UA (A and B) and UV (D and E). Sections of the blood vessels from normal term delivered placentas with umbilical cord were examined. Omission of the primary polyclonal antibodies served as the negative control (CTL; C and F). E, endothelial cell; SMC, smooth muscle cell. Original magnification, ×40.
**Statistical analysis.** Data are presented means ± SE. Relaxation to CGRP was expressed as a percentage of the initial precontraction to 5-HT or U-46619. Raw data for individual concentration-response curves were also compared by two-way repeated-measures ANOVA. The Bonferroni-Dunn post hoc test was used for determining significant differences between factors. Student’s unpaired t-test was used for statistical comparison of logEC₅₀ values. P values < 0.05 were considered statistically significant.

**RESULTS**

**mRNA expression for CRLR/RAMP₁.** To examine whether the CGRP-A receptor components CRLR and RAMP₁ are present in human fetoplacental tissues, we analyzed mRNA expression by RT-PCR using the specific primers. As shown in Fig. 1, mRNA encoding both CRLR and RAMP₁ was expressed in the human umbilical artery and vein, chorionic artery and vein, and stem villous artery and vein, suggesting the existence of CGRP-A receptors in the human fetoplacental unit. After the expressions of CRLR and RAMP₁ were normalized to 18S RNA, no significant differences in CRLR and RAMP₁ mRNA expression were observed among all the vessels examined, implying homogenous levels of expression of these CGRP-A receptor components in the fetoplacental vasculature.

**Protein expression for CRLR/RAMP₁.** Western blotting with polyclonal antibodies shows single bands of proteins for CRLR at 53 kDa and RAMP₁ at 14 kDa in the human umbilical artery and vein, chorionic artery and vein, and stem villous artery and vein (Fig. 2). This set of data is parallel to the mRNA data, confirming the existence of CGRP-A receptors in the human fetoplacental unit. Again, there were no significant differences in CRLR and RAMP₁ protein expression among the various vessels.

---

Fig. 4. Immunofluorescent localization of CRLR (A and D) and RAMP₁ (B and E) in the human CA (A and B) and CV (D and E). Sections of the blood vessels from normal term delivered placental were examined. Omission of the primary polyclonal antibodies served as the negative control (C and F). E, endothelial cell. Original magnification, ×40.
Cellular localization of CRLR and RAMP₁. Using immunofluorescent staining, we found that both CRLR and RAMP₁ were present in the umbilical artery and vein (Fig. 3), with staining localized to both the endothelium and underlying smooth muscle cells, implying that CGRP could exert its effects in the control of umbilical vascular tone. Control sections without primary antibody for both CRLR and RAMP₁ showed no specific staining in the umbilical segments. Specific staining for CRLR and RAMP₁ was also present in the chorionic artery and vein (Fig. 4), with staining localized to the endothelium and underlying smooth muscle layer. Immunostaining of placental villi showed that, in addition to endothelial and smooth muscle cells, the trophoblast layer demonstrated intense staining for both CRLR and RAMP₁ (Fig. 5). These results show the existence of CGRP-A receptors in not only blood vessels but also in the trophoblast cells of the placenta.

Vasodilatory property of CGRP. To examine the vasodilatory effects of CGRP in the fetoplacental circulation, the rings of umbilical and chorionic plate vessels were mounted in an organ bath and the changes in isometric tension were recorded and analyzed with the DATAQ system. CGRP dose dependently relaxed 5-HT (5 × 10⁻⁷ M)-preconstricted umbilical arteries and veins (Fig. 6). 5-HT-induced vascular contraction in both the artery and vein was maintained in the absence of CGRP and served as a temporal control. Similar to the effects in umbilical vessels, CGRP dose dependently relaxed 5-HT-preconstricted chorionic arteries and veins (Fig. 7). Summarized data from six patients are plotted in Fig. 8. Compared with umbilical arteries and chorionic arteries, the stem villous arteries displayed a further relaxation at 1 × 10⁻⁷ and 1 × 10⁻⁶ M CGRP. These changes were confirmed by the analysis of logEC₅₀ of CGRP (Table 2), implying an increased sensitivity to CGRP in stem villous vessels.
The relaxation effects of CGRP on the chorionic artery were profoundly inhibited by CGRP 8–37, a specific antagonist of CGRP receptors (Fig. 9). Data shown in Fig. 10 with six samples measured in each point further confirmed that CGRP 8–37 blocked the vasodilatory effect of CGRP, indicating that CGRP relaxant actions in the fetoplacental vessels are mediated via CGRP receptors. 

**Postreceptor signaling pathway of CGRP-induced vascular relaxation.** To further examine the postreceptor signaling pathway of CGRP-induced relaxation in human fetoplacental vessels, we incubated the vascular rings with different reagents before the application of CGRP. As shown in Fig. 11, incubation of the chorionic artery with glibenclamide, an KATP channel blocker, and Rp-cAMPS, a cAMP-dependent protein kinase A inhibitor, completely blocked CGRP-induced vascular relaxation. l-NAME, a nitric oxide synthase inhibitor, on the other hand, partially inhibited CGRP-induced vascular relaxation. Furthermore, the blockade of CGRP-induced fetoplacental vascular relaxation with various drugs was demonstrated by the analysis of logEC50 of CGRP in the human chorionic artery (Table 3). The logEC50 of CGRP in chorionic artery relaxation was significantly increased by glibenclamide (−7.93 ± 0.03), Rp-cAMPS (−7.93 ± 0.03), and l-NAME (−8.20 ± 0.06) compared with the control (−8.35 ± 0.02, P < 0.05 by ANOVA). These demonstrations imply that the activation of KATP channels, the accumulation of cAMP, and, to a lesser extent, the production of nitric oxide are probably involved in the CGRP signaling pathway.

**DISCUSSION**

The maintenance of adequate blood flow to the placenta is important to fetal growth and development. Impaired placental circulation may eventually lead to intrauterine growth restriction, which is a significant complication of pregnancy. Medical and obstetrical conditions may alter the fetoplacental vascular flow and responsiveness to various substances. Therefore, the examination of the role of the potent vasodilator CGRP in the fetoplacental circulation is important to understand the mechanisms of the control of fetoplacental vascular tone under normal and diseased conditions. The present study provides solid evidence to demonstrate that 1) CRLR and RAMP1...
mRNA and proteins are abundantly expressed in the human fetoplacental unit; 2) CRLR and RAMP1 proteins are primarily localized in the vascular endothelium and underlying smooth muscle cells as well as in the trophoblast layer of the placental villi; 3) CGRP dose dependently relaxed fetoplacental vessels in vitro, and these effects are primarily mediated via CGRP receptors; 4) the stem villous artery layer is especially sensitive to the relaxation effects of CGRP; and 5) vascular dilations in response to CGRP appear to involve the activation of K_ATP channels, cAMP production, and a nitric oxide pathway. Thus we conclude that CGRP-induced vasodilation in the fetoplacental unit may contribute to the low vascular resistance and fetal growth during normal pregnancy.

Fetal growth and well-being depends mainly on uteroplacental and fetoplacental blood flow, which must be adequate to ensure exchanges between the maternal and fetal compartment through the placental barrier. Potential sites for vascular resistance control in the fetoplacental circulation have been widely investigated. In the human, it has been demonstrated that umbilical arterial blood flow depends on the pressure gradient that drives blood flow from the fetal interior vena cava to the human iliac artery and on the total vascular resistance of the serially arranged fetoplacental vessel pathway (1). In most systemic vascular beds, large arteries and veins contribute little to the total vascular resistance of the circulation and therefore are not important to the control of organ blood flow. However, in the human fetoplacental circulation, the umbilical artery and vein are extremely long, and the resistance of the placental microcirculation is extremely low (1). As we know in the sheep model, almost one-half of the total placental vascular resistance resides in the umbilical vessels and their major branches (35). In the human, the umbilical vessels are on average of four times longer than those in the sheep. Therefore, umbilical vessels and chorionic plate vessels in the human may make even more of a contribution to total fetoplacental vascular resistance (1). The present study demonstrated for the first time that mRNA and protein for CRLR and RAMP1 are abundantly expressed in the human fetoplacental vasculature, and they are primarily localized in the vascular endothelium and underlying smooth muscle cells, suggesting that CGRP may play an important role in the control of fetoplacental vascular tone and thus the modification of local vascular resistances.

Similar to the vascular endothelial cells and smooth muscle cells, the trophoblast cells also expressed CRLR and RAMP1 proteins, indicating that CGRP may play a role in the regulation of trophoblast functions. It is well known that in early pregnancy and during implantation, trophoblast cells act as the

### Table 2. LogEC50 of CGRP in human fetoplacental vessels

<table>
<thead>
<tr>
<th>Vessels</th>
<th>logEC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Umbilical artery</td>
<td>−8.33±0.02</td>
</tr>
<tr>
<td>Chorionic artery</td>
<td>−8.35±0.02</td>
</tr>
<tr>
<td>Stem villous artery</td>
<td>−8.43±0.05*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 arteries/group. *P < 0.05 vs. the umbilical artery by ANOVA.
leading edge of embryo invasion of the maternal endometrium (10). Trophoblast cells are an important immunological barrier protecting the embryo from the maternal immune response (25). Furthermore, trophoblast cells exert endocrine functions, synthesizing and secreting steroid and peptide hormones (40). The present study indicates a possible link between CGRP and trophoblast cell functions. Apparently, additional studies examining the effects of CGRP on trophoblast invasion and angiogenesis are required to fully understand the role of CGRP in early pregnancy and implantation.

The human placenta responds to various vasoactive substances and neuropeptides, which may play an important role in the local regulation of fetoplacental blood flow in both the maternal and fetal compartments, and thus is critical for fetal growth and development (21). Few studies have directly examined the role of CGRP on human fetoplacental circulation. Some reports have shown a concentration-dependent vasodilation of CGRP in dually perfused cotyledon (29). Although those observations suggested that CGRP is a vasodilator in placental vessels, the mechanisms of CGRP-induced vasodilation are not defined. The present study demonstrated that CGRP dose dependently relaxes fetoplacental vessels in vitro, and the vascular dilations in response to CGRP are similar between the umbilical artery and vein and chorionic artery and vein, suggesting similarities in responsiveness to CGRP. These may be explained by the evenly distributed CRLR and RAMP1 in the fetoplacental vasculature, as evidenced by mRNA expression, protein expression, and immunofluorescent staining. Meanwhile, we noted that compared with the umbilical artery and chorionic artery, the stem villous artery displayed a further relaxation to CGRP at $10^{-7}$ to $10^{-6}$ M, implying increased sensitivity to CGRP in stem villous vessels. We (15) have recently reported the expression and regulation of CGRP-B receptor in the rat placenta. We postulated that, in addition to the CGRP-A receptor, the CGRP-B receptor may also exist in the human fetal placental vasculature to mediate CGRP-induced vascular relaxation. The distribution and regulation of CGRP-B receptor in the human placenta warrant further investigation.

CGRP$_{8-37}$ is a competitive inhibitor to CGRP binding (38). Radioligand studies and functional analysis have demonstrated that CGRP receptors display the highest sensitivity to CGRP$_{8-37}$ (23). We (19) have previously reported that acute administration of CGRP$_{8-37}$ significantly increases mean arterial pressure in l-NAME-treated pregnant rats. Recently, new data from our group showed that chronic administration of CGRP$_{8-37}$ to pregnant rats caused a significant reduction in pup weight and increases in systolic blood pressure and fetal mortality rate, and these effects were dose dependent (18). CGRP$_{8-37}$ has been shown to attenuate CGRP-induced vasodilation in vitro perfused human placental cotyledons (29). In the present study, the relaxation effects of CGRP on human fetoplacental vessels were profoundly inhibited by CGRP$_{8-37}$, further confirming that vasodilatory effects of this peptide is mediated by CGRP receptors.

Depending on the vascular bed and the phenotype of the receptive cell, the mechanisms of CGRP action may vary. CGRP activates adenylate cyclase and elevates cellular levels of cAMP in a number of cell types. CGRP-induced relaxation in both circular and longitudinal intestinal smooth muscle cells of guinea pig ileum involves cAMP production and nitric oxide release (39). In cultured rat aortic smooth muscle cells, CGRP induced accumulation of cAMP, and this accumulation was enhanced by the nitric oxide donor sodium nitroprusside (27). In the isolated porcine coronary artery, CGRP-induced relax-

![Fig. 10. Blockade of CGRP-induced CA relaxation by CGRP$_{8-37}$ (10$^{-5}$ M). Relaxation responses, expressed as a percentage of control activity at each dose of CGRP, were analyzed by two-way repeated-measures ANOVA between the two groups (6 patients each). *Significant difference vs. CGRP$_{8-37}$ (P < 0.05).

![Fig. 11. CGRP dose-dependent curves for relaxation of 5-HT-induced contracting CAS. Relaxation responses, expressed as a percentage of control activity at each dose of CGRP, were analyzed by two-way repeated-measures ANOVA among the four groups (6 patients each). The vascular segments were incubated for 30 min in fresh Krebs solution with either saline (CTL), glibenclamide (Glib; 10$^{-5}$ M), Rp-cAMPS (10$^{-5}$ M), or N$^\text{G}$-nitro-L-arginine methyl ester (L-NAME; 10$^{-5}$ M). *Significant difference in CTL vs. Glib and Rp-cAMPS (P < 0.05) and in CTL vs. L-NAME (P < 0.05).](http://ajpheart.physiology.org/)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LogEC$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>–8.35±0.02</td>
</tr>
<tr>
<td>CGRP$_{8-37}$</td>
<td>–7.93±0.23*</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>–7.93±0.03*</td>
</tr>
<tr>
<td>Rp-cAMPS</td>
<td>–7.92±0.03*</td>
</tr>
<tr>
<td>L-NAME</td>
<td>–8.20±0.06*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 arteries/group. L-NAME, N$^\text{G}$-nitro-L-arginine methyl ester. *P < 0.05 vs. control by ANOVA.
vation was accompanied by increases in cAMP (44). In human colon smooth muscle cells, CGRP induces relaxation via both cGMP and cAMP production (6). The present study demonstrated that pretreatment of human chorionic arteries with the Rp diastereomer of adenosine cyclic 3',5'-phosphorothioate, Rp-cAMPS, which is a novel membrane-permeable antagonist of cAMP (11), completely inhibits CGRP-induced vascular relaxation, suggesting that KATP channels exist in human rabbits (3). In agreement with these observations, CGRP-induced increases in pulmonary blood flow resistance were accompanied by increases in cAMP (44). In human chorionic arteries, CGRP-induced relaxation was accompanied by increases in cAMP (44). In human skeletal muscle arteries and pulmonary arteries and veins (31, 36). Although the present study did not directly address the role of the endothelium in CGRP-induced fetoplacental vasodilation, our data showing that L-NAME partially inhibited CGRP actions imply that KATP channels exist in human fetoplacental vessels and that these channels were involved in CGRP-induced fetoplacental vascular relaxation.

The potency of CGRP’s vasoreactivity and the requirement for the presence of intact endothelium has a marked regional variation. Endothelium is absolutely required for CGRP actions in the rat thoracic aorta and renal and pulmonary arteries and in the human cerebral arteries (20, 22). On the other hand, endothelium-independent vasorelaxation was demonstrated in response to CGRP in human skeletal muscle arteries and pulmonary arteries and veins (31, 36). Although the present study did not directly address the role of the endothelium in CGRP-induced fetoplacental vasodilation, our data showing that L-NAME partially inhibited CGRP actions imply that stimulation of nitric oxide synthesis may also contribute to the CGRP-induced vasodilation. In view of the above, pretreatment of human chorionic arteries with the RP diastereomer of adenosine cyclic 3',5'-phosphorothioate, RP-cAMPS, which is a novel membrane-permeable antagonist of cAMP (11), completely inhibits CGRP-induced vascular relaxation, suggesting that KATP channels exist in human chorionic arteries (3).

CGRP has been demonstrated to activate KATP channels via cAMP-dependent protein kinase. In rabbit mesenteric arteries (37), CGRP stimulates adenyl cyclase, which leads to an elevation of cAMP. In turn, cAMP activates protein kinase A, which opens KATP channels. In osteoblastic UMR 106 cells, CGRP-induced membrane hyperpolarization in a dose-dependent manner (26); this membrane hyperpolarization was totally antagonized by glibenclamide, a selective KATP channel blocker, indicating the involvement of KATP channel in CGRP action. Glibenclamide has been also demonstrated to antagonize CGRP-induced increases in pulmonary blood flow in fetal sheep (43) and attenuate CGRP-induced hypotension in the rabbits (3). In agreement with these findings, the present study showed that glibenclamide abolished CGRP-induced vascular relaxation, suggesting that KATP channels exist in human fetoplacental vessels and that these channels were involved in CGRP-induced fetoplacental vascular relaxation.

ACKNOWLEDGMENTS

We thank Kimberly Mitchell for excellent typing work.

REFERENCES

6. Boyer JC, Christen MO, Balmes JL, and Bali, JP. Calcitonin gene-related peptide- and L-NAME partially inhibited CGRP actions imply that KATP channels exist in human fetoplacental vessels and that these channels were involved in CGRP-induced fetoplacental vascular relaxation.

The potency of CGRP’s vasoreactivity and the requirement for the presence of intact endothelium has a marked regional variation. Endothelium is absolutely required for CGRP actions in the rat thoracic aorta and renal and pulmonary arteries and in the human cerebral arteries (20, 22). On the other hand, endothelium-independent vasorelaxation was demonstrated in response to CGRP in human skeletal muscle arteries and pulmonary arteries and veins (31, 36). Although the present study did not directly address the role of the endothelium in CGRP-induced fetoplacental vasodilation, our data showing that L-NAME partially inhibited CGRP actions imply that stimulation of nitric oxide synthesis may also contribute to the CGRP-induced vasodilation. In view of the above, pretreatment of human chorionic arteries with the RP diastereomer of adenosine cyclic 3',5'-phosphorothioate, RP-cAMPS, which is a novel membrane-permeable antagonist of cAMP (11), completely inhibits CGRP-induced vascular relaxation, suggesting that KATP channels exist in human chorionic arteries (3).

CGRP has been demonstrated to activate KATP channels via cAMP-dependent protein kinase. In rabbit mesenteric arteries (37), CGRP stimulates adenyl cyclase, which leads to an elevation of cAMP. In turn, cAMP activates protein kinase A, which opens KATP channels. In osteoblastic UMR 106 cells, CGRP-induced membrane hyperpolarization in a dose-dependent manner (26); this membrane hyperpolarization was totally antagonized by glibenclamide, a selective KATP channel blocker, indicating the involvement of KATP channel in CGRP action. Glibenclamide has been also demonstrated to antagonize CGRP-induced increases in pulmonary blood flow in fetal sheep (43) and attenuate CGRP-induced hypotension in the rabbits (3). In agreement with these findings, the present study showed that glibenclamide abolished CGRP-induced vascular relaxation, suggesting that KATP channels exist in human fetoplacental vessels and that these channels were involved in CGRP-induced fetoplacental vascular relaxation.

Normal pregnancy is associated with an increase in uteroplacental and fetoplacental blood flow and a decrease in uterine and placental vascular resistance (28, 41). The direct impairment of uterine and placental arterial flow results in intrauterine growth restriction and low birth weight, which in turn correlates with neonatal morbidity and mortality (5, 24). The present study was deliberately performed with placentas from uncomplicated pregnancies to describe the fetoplacental vascular response to CGRP under normal conditions. Further studies are apparently required to examine whether any alterations in the fetoplacental vascular response to CGRP and the expression of CGRP receptors and postreceptor mechanisms in placentas from pregnancies with various pathological conditions, such as intrauterine growth restriction and/or pre-eclampsia.

ACKNOWLEDGMENTS

We thank Kimberly Mitchell for excellent typing work.

GRANTS

This study was supported by National Institutes of Health Grants HD-38324, HL-70883, and HL-58144.

REFERENCES

6. Boyer JC, Christen MO, Balmes JL, and Bali, JP. Calcitonin gene-related peptide and L-NAME partially inhibited CGRP actions imply that KATP channels exist in human fetoplacental vessels and that these channels were involved in CGRP-induced fetoplacental vascular relaxation.

The potency of CGRP’s vasoreactivity and the requirement for the presence of intact endothelium has a marked regional variation. Endothelium is absolutely required for CGRP actions in the rat thoracic aorta and renal and pulmonary arteries and in the human cerebral arteries (20, 22). On the other hand, endothelium-independent vasorelaxation was demonstrated in response to CGRP in human skeletal muscle arteries and pulmonary arteries and veins (31, 36). Although the present study did not directly address the role of the endothelium in CGRP-induced fetoplacental vasodilation, our data showing that L-NAME partially inhibited CGRP actions imply that stimulation of nitric oxide synthesis may also contribute to the CGRP-induced human fetoplacental vascular relaxation.

Normal pregnancy is associated with an increase in uteroplacental and fetoplacental blood flow and a decrease in uterine and placental vascular resistance (28, 41). The direct impairment of uterine and placental arterial flow results in intrauterine growth restriction and low birth weight, which in turn correlates with neonatal morbidity and mortality (5, 24). The present study was deliberately performed with placentas from uncomplicated pregnancies to describe the fetoplacental vascular response to CGRP under normal conditions. Further studies are apparently required to examine whether any alterations in the fetoplacental vascular response to CGRP and the expression of CGRP receptors and postreceptor mechanisms in placentas from pregnancies with various pathological conditions, such as intrauterine growth restriction and/or pre-eclampsia.

ACKNOWLEDGMENTS

We thank Kimberly Mitchell for excellent typing work.

GRANTS

This study was supported by National Institutes of Health Grants HD-38324, HL-70883, and HL-58144.


