Contractile properties of human placental anchoring villi

Anne E. Farley,1 Charles H. Graham,2 and Graeme N. Smith1,2
Departments of 1Obstetrics and Gynecology and 2Anatomy and Cell Biology, Queen’s University, Kingston, Ontario, Canada K7L 3N6
Submitted 1 April 2004; accepted in final form 10 May 2004

Farley, Anne E., Charles H. Graham, and Graeme N. Smith. Contractile properties of human placental anchoring villi. Am J Physiol Regul Integr Comp Physiol 287: R680 –R684, 2004. First published May 13, 2004; 10.1152/ajpregu.00222.2004.—The presence of myofibroblasts arranged parallel to the longitudinal axes of anchoring villi of the placenta has previously been described. Furthermore, it has been suggested that intraplacental blood volume, and hence fetal-maternal oxygen-nutrient exchange, may in part be regulated through the longitudinal contraction of anchoring villi. We demonstrate here that anchoring villi have the ability to contract and relax longitudinally. Anchoring villi from normal term human placenta were dissected and suspended from force-displacement transducers to determine their longitudinal contractility in response to potassium chloride (KCl), Nω-nitro-l-arginine methyl ester (l-NAME) and the nitric oxide donors sodium nitroprusside (SNP) and glyceryl trinitrate (GTN). Treatment with both KCl and l-NAME resulted in up to a 62% and 74% increase, respectively, in longitudinal contraction over resting tone. In contrast, both SNP and GTN caused a dose-dependent relaxation of precontracted villi. Immunohistochemistry of longitudinal sections of villi confirmed the presence of α-actin-containing cells in the extravascular space. Histological staining with hemotoxylin and eosin confirm that the tissue used in these experiments were anchoring villi. These findings suggest that the contraction of anchoring villi may be an important mechanism whereby the placenta may regulate intraplacental volume.

THE ANCHORING VILLI (Fig. 1) of the human placenta are treelike structures made up of a primary trunk attached to the chorionic plate, secondary branches, and tertiary, very fine “anchoring” branches attached to the basal plate (2). Histochemically and ultrastructurally, smooth muscle cells and myofibroblasts have been identified in human anchoring villi (11). They also showed that a strip of tissue dissected from an anchoring villus was capable of lengthening and shortening in response to calcium chloride, potassium chloride, and barium chloride (11). It has been shown that myosin, the major contractile protein, is present in large amounts within the “extravascular contractile system” (5) of the anchoring villi (10). The innermost layer of longitudinally oriented extravascular stromal cells of the villi are immunoreactive to all five of the characteristic cytoskeletal proteins for smooth muscle cells (vimentin, desmin, α- and γ-actin, and myosin) (4). In contrast to smooth muscle cells of the tunica media of blood vessels, cells in the extravascular contractile system of anchoring villi also express dipetidylpeptidase IV [a known marker for the extravascular contractile system in stem villi (8)]. Although the evidence for the presence of cells capable of contracting along the longitudinal axes of anchoring villi is strong, little research has been carried out demonstrating its physiological significance. The purpose of this study was to determine whether anchoring villi are capable of contraction/relaxation in an isolated tissue bath and to characterize the underlying mechanisms involved.

MATERIALS AND METHODS

Term human placentae from uncomplicated pregnancies were obtained immediately after cesarean section or vaginal delivery at the Kingston General Hospital, Kingston, Ontario, Canada. Anchoring villi were isolated by gentle dissection from the chorionic and basal plates. Villi were rinsed in Krebs solution to remove excess blood. The villi were tied at both ends by silk suture, suspended from a force-displacement transducer, and placed in an isolated tissue bath in 15 ml of standard Krebs solution at 37°C. Tissues baths were bubbled with 95% or 21% O2, 5% CO2, balance N2. In all experiments, the force-displacement transducer (Grass FTO3D) coupled to a Powerlab (ADInstruments) was calibrated to a maximum of 2 g of tension. Placental arterial rings of ~3 mm in length were used as positive controls for contraction. The arterial ring was suspended from two stainless steel hooks inserted through the lumen of the ring. On the basis of preliminary studies, the tissue was set to 1.5-g resting tension and allowed to equilibrate in the tissue baths for 1 h with rinses of fresh oxygenated Krebs solution every 15 min.

Potassium chloride dose response. After equilibration, the resting tension was adjusted to 1.5 g and 0.12 mol/l KCl was added to each bath to precontract the villi (n = 6). The villi were allowed to equilibrate for 15 min and then rinsed three times in 15-min increments for a total of 1 h. KCl (0.015, 0.03, and 0.06 mol/l) was added to each bath and allowed to equilibrate for 15 min.

l-NAME dose response. Villi (n = 17) were suspended as previously described but not precontracted with KCl treatment. l-NAME (l-NAME, Sigma-Aldrich) was added in increasing concentrations (25–200 μmol/l) as an accumulating dose to the tissue baths.

Sodium nitroprusside/glyceryl trinitrate dose response. The villi were maximally precontracted with KCl 0.06 mol/l. Sodium nitroprusside (SNP; Sigma-Aldrich) in five increasing concentrations (0.7–700 μmol/l), as an accumulating dose without rinsing, was added to the baths in 15-min increments (n = 6). In a separate set of experiments, glycerol trinitrate (GTN; Tridil, Sabex, Boucherville, QC) in five increasing concentrations (1–15 mmol/l), as an accumulating dose, was added to the baths in 15-min increments (n = 13). After the final concentrations were added and allowed to equilibrate, the villi were rinsed, allowed to equilibrate, and again 0.06 mol/l KCl was added to the bath to confirm tissue viability by contraction.

Tissue preparation. At the conclusion of the experiments, the villi were weighed and processed for histology and immunostaining for α-actin as previously described (12). Paraffin-embedded tissue was cut into 5-μm-thick serial sections. For α-actin immunohistochemistry, primary polyclonal antibody anti-α-actin (A2066; Sigma-Aldrich) was applied to the sections overnight at a dilution of 1:50 in PBS buffer. Antigen was detected using a biotinylated anti-rabbit IgG secondary antibody (1:200 dilution) and avidin-biotin-peroxidase (Vector

Address for reprint requests and other correspondence: G. N. Smith, Dept. of Obstetrics and Gynecology, Kingston General Hospital, 76 Stuart St., Kingston, Ontario, Canada K7L 2V7 (E-mail: gns@post.queensu.ca).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

R680
0363-6119/04 $5.00 Copyright © 2004 the American Physiological Society http://www.ajpregu.org
Laboratories, Burlington, Ontario, Canada) followed by precipitation of diaminobenzidine chloride (Dako, Mississauga, Ontario, Canada). Fresh tissue was processed and stained with hemotoxylin and eosin (H&E) for histological identification of an anchoring villus (Fig. 1).

**Statistical analysis.** Data were analyzed with repeated measures one-way ANOVA and post hoc Newman-Keuls test for multiple comparisons between group means using GraphPad Prism 3.0 (GraphPad Software, San Diego, CA). Results were statistically significant if $P < 0.05$. All results are stated as means with SD in parentheses.

**RESULTS**

The villi exhibited contraction in a dose-dependent manner to increasing concentrations of KCl, with significance obtained at KCl concentrations of 0.015 mol/l and 0.06 mol/l (Figs. 2 and 3, $P < 0.05, n = 6$). KCl increased tension from 20% (12.0 SD) to 62.3% (34.5 SD) of maximal contraction. The cumulative L-NAME administration caused contraction of the tissue from its resting state and the results were statistically significant (Fig. 4, $P < 0.05, n = 17$). L-NAME was capable of increasing tension from 63.5% (19.1 SD) to 74.3% (21 SD) above resting state. SNP produced a dose-dependent relaxation of the precontracted villi over the cumulative concentration range that was statistically significant between groups (Figs. 5 and 6, $P < 0.05, n = 6$). SNP caused a decrease in maximal tension of 4.8% (5.5 SD) to 58.1% (23.5 SD). GTN induced relaxation of 20.2% (44.0 SD) to 73.4% (51.0 SD) (Fig. 7, $P < 0.05, n = 13$). Immunostaining of anchoring villi demonstrated the presence of $\alpha$-actin throughout the longitudinal axis of the extravascular villous tissue of the primary villus trunk (Fig. 8).
DISCUSSION

Our study demonstrates that human chorionic villi are capable of contracting along their longitudinal axes. Specifically, the results revealed that KCl, an agent that depolarizes the cell and causes the release of intracellular calcium via a receptor-independent mechanism, was able to reversibly contract the villi. Furthermore, pharmacological blockade of nitric oxide (NO) production with the NO synthase (NOS) antagonist L-NAME also induced contraction of the villi, thus indicating that NO-mediated signaling plays a role in regulating villous tone. In support of this concept are the results of experiments in which two classes of NO mimetic agents, namely SNP and GTN, were able to induce relaxation of KCl precontracted villi. The relatively high dose of L-NAME required to produce the observed effect likely reflects the layers of tissue needed to penetrate to the villi to inhibit NO production and result in longitudinal contraction.

Smooth muscle-like spindle-shaped cells were first observed in the chorionic plate of the term human placenta in 1906 (11). However, it was not until 1916 that Izuka (11) confirmed their smooth muscle cell identity. Also noted at that time was that these muscle cells were denser at the center of the placenta where the umbilical cord inserted and more sparse toward the periphery. In 1922, Naujoks (11) proposed the hypothesis that the human placenta is capable of contraction and Krantz and Arey (11) later demonstrated that these cells in the placenta have all the morphological characteristics of smooth muscle. More recently, Graf et al. (5) confirmed the presence of myofibroblasts in stem villi by demonstrating the immunolocalization of vimentin, desmin, α-actin, γ-actin, and myosin in these cells (5). Those investigators also proposed that the change in villus length is an active process (5). Using a similar approach, we confirmed previous findings in which α-actin was shown to be present in longitudinally oriented cells within the extravascular stroma of the primary trunk of anchoring villi (4, 6). Graf et al. (7) also identified a system of elastic-collagen fibers running longitudinally along the fetal vessels of the placenta and confirmed that all three elements of elastic tissue were present in human placenta. Thus the demonstration of contractile elements in anchoring villi supports the hypothesis that the placenta has the ability to regulate its own intravillous volume. This may in fact be a combination of longitudinal contraction/relaxation of both the extravascular contractile system of anchoring villi and the smooth muscle cells of the tunica media of blood vessels, resulting in an overall shortening/lengthening of the anchoring villi within the placenta. The contraction/relaxation of the villi may impact on both maternal perfusion and fetal villous blood flow and thus contribute to maternal-fetal perfusion matching. For example, increase in fetal oxygen/nutrient demands may result in increased maternal/intravillous placental flow as a function of relaxation of the anchoring villi. A failure of this effect in regulating placental perfusion may play a role in adverse obstetrical outcomes of...
placental origin, such as intrauterine growth restriction. Similarly, as preeclampsia is thought to be related to abnormalities of placental perfusion (perhaps regionally), this may be secondary to an inherent defect in the placenta itself or contribute to poor maternal perfusion because of impaired spiral artery invasion.

The mechanisms controlling villous contractions are not fully characterized. As our findings indicate, locally produced NO may be involved in this process. Carbon monoxide (CO), a molecule produced during the breakdown of heme through a reaction catalyzed by the enzyme heme oxygenase (HO), may also play a role in this regulation. There is evidence that CO is capable of inducing vascular relaxation through a mechanism that, in a similar manner to NO, involves activation of soluble guanylyl cyclase and generation of cGMP (1, 9). Whereas in the present study both SNP and GTN induced villus relaxation, the concentrations required to induce significant relaxation were much lower for SNP than for GTN. One possible explanation for this is that GTN is a prodrug that requires biotransformation, whereas SNP releases NO more readily in solution. It is unknown if the contractile elements of anchoring villi are more/less sensitive to vasodilators/constrictors compared with vascular smooth muscle. In Bobier et al. (3), rings of chorionic plate vessels (CPV) were precontracted with 0.12 M KCl and submaximally contracted (30–50% of max) with 0.03 M KCl; the same concentrations of KCl achieved similar results in the anchoring villi. Relaxation was achieved with SNP in the CPV rings using concentration ranges of 10^{-12} to 10^{-1} M, whereas SNP in concentrations that were six magnitudes higher (0.7 \times 10^{-6} to 0.7 \times 10^{-3}) were required to cause relaxation in the tissue. The observation of greater drug concentrations required in the present study likely relates to the need to diffuse across a larger tissue mass to get to the site of action. In summary, our findings demonstrate that anchoring villi are capable of contraction/relaxation. This may represent a novel mechanism by which the placenta controls its own volume and thereby maintains adequate nutrient and oxygen delivery as well as participating in maternal-fetal perfusion matching.

ACKNOWLEDGMENTS

We give our thanks to Dr. K. Nakatsu for assistance with interpretation of the data and Dr. P. Kaufman for interest in this work.

GRANTS

This work was supported by a grant from the Canadian Institutes of Health Research (Grant #MOP64305) and the Heart and Stroke Foundation of Ontario (Grant T466). G. N. Smith is the recipient of a New Investigator Award from the Canadian Institutes of Health Research.

Fig. 7. Effect of glyceryl trinitrate (GTN) on precontracted anchoring villi (n = 13). GTN was added in cumulative increasing concentrations. Group means with different letters are statistically significant from each other (P < 0.05). Bars represent the mean percent change (±SD) in contractile force compared with maximal contraction.

Fig. 8. Immunostaining with anti-α-actin and diaminobenzidine chloride (DAB) peroxidase substrate (dark brown). Bars represent 100 μm. A: positive arterial ring shows concentrated staining in the media of the vessel. B: control arterial ring, no anti-α-actin staining. C: longitudinal section of the primary villus trunk of an anchoring villus positive for α-actin shows staining in the extravascular contractile system. D: boxed area of C shows detailed staining of longitudinal cells in the extravascular contractile system.
REFERENCES


