BIOLOGY OF REPRODUCTION (2015) **93**(6):134, 1–10
Published online before print 28 October 2015.
DOI 10.1095/biolreprod.115.135665

Pregnancy Increases Relaxation in Human Omental Arteries to the CGRP Family of Peptides[1]

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ABSTRACT

Calcitonin gene-related peptide (CALCB) and its family members adrenomedullin (ADM) and intermedin (ADM2) play important roles in maintaining vascular adaptations during pregnancy in animal models. The present study was designed to evaluate the responses of omental arteries to CALCB, ADM, and ADM2 in pregnant and nonpregnant women, and to determine the mechanisms involved. By using resistance omental arteries collected from nonpregnant women (n = 15) during laparotomy and from term pregnant women (n = 15) at cesarean delivery, this study shows that the receptor components—calcitonin receptor-like receptor (CALCRL) and receptor activity-modifying proteins (RAMPs) 1, 2 and 3—are localized to endothelial and smooth muscle cells in omental arteries, with increased expressions of both mRNA and protein in pregnant compared with nonpregnant women. The myography study demonstrated that CALCB, ADM, and ADM2 (0.1–100 nM) dose dependently relax U46619 (1 muM) precontracted omental artery segments, and the maximum possible effects to CALCB and ADM2, but not to ADM, are significantly enhanced in pregnant compared with nonpregnant women. Further, the vasodilatory responses to CALCB, ADM, and ADM2 are reduced by inhibitors of nitric oxide (NO) synthase (L-NAME), adenylyl cyclase (SQ22536), voltage-activated potassium channels (4-aminopyrodin and tetrabutylammonium), Ca^{2+}-activated potassium channel (charybdotoxin), and cyclooxygenase (indomethacin). In conclusion, the CALCB family of peptides, CALCB and ADM2, increase human omental artery relaxation during pregnancy through diverse mechanisms, including NO, endothelium-derived hyperpolarizing factors (EDHFs) and prostaglandins, and thus could contribute to the vascular adaptations during pregnancy in the human.

CALCB family peptides, human omental artery, pregnancy

INTRODUCTION

Normal pregnancy is characterized by pronounced systemic vascular adaptations, including marked increases in total blood volume, cardiac output, and heart rate, as well as decreased peripheral vascular resistance and mean arterial pressure [1]. These vascular adaptations are believed to be dependent on enhanced endothelium-dependent dilatation [1, 2] and circulatory vasoactive agents [1, 3, 4]. However, the molecular mechanisms underlying these changes are not fully understood. It has been suggested that the vasodilatory capacity of the endothelium is achieved by the combined effects of nitric oxide (NO), endothelium-derived hyperpolarizing factors (EDHFs), and prostacyclin (PGI_2). The contribution of these endothelium-derived substances varies among vessels of different sizes, the vascular bed studied, the agonist tested, and perhaps the species. Recent studies in rats have suggested that calcitonin gene-related peptide (CALCB) and its family members, adrenomedullin (ADM) and intermedin (ADM2), play an important role in maintaining vascular adaptation during pregnancy [4, 5]. However, it is unknown whether these peptides play a role in the gestation-related vascular adaptations in the human.

CALCB, a 37-amino acid peptide primarily synthesized in the sensory neurons of dorsal root ganglia, is one of the most potent endogenous vasodilators known [6]. CALCB levels are significantly increased in both the maternal and fetal serum of women [7], and in rat serum [8, 9], compared with the nonpregnant state. ADM, a 52-amino acid peptide, is produced by a wide range of cells, including vascular endothelial and smooth muscle cells [10]. Plasma concentrations of ADM are elevated during pregnancy both in humans [11, 12] and in rats [13]. ADM2 is a 47-amino acid peptide with 28% structural homology to ADM and 20% to CALCB. ADM2 is abundantly expressed in rat ovary, uterus, stomach, and kidney [14], and the plasma levels of ADM2 are elevated in pregnant rats [15]. In rats, all three peptides exert significantly greater vasodilatory potency during pregnancy compared with the nonpregnant state [4, 15–17].

CALCB, ADM, and ADM2 exert biological functions through their overlapping receptor components, calcitonin receptor-like receptor (CALCRL), and receptor activity-modifying proteins (RAMP1, RAMP2, and RAMP3) [4, 14, 18]. It is well accepted that coexpression of CALCRL with RAMP1 forms a CALCB receptor, whereas RAMP2 or RAMP3 produces an ADM receptor, and coexpression of CALCRL with any of the three RAMPs mediates ADM2 signaling. Expression of these receptor components has been reported in several vascular beds of animals and humans.

Omental arteries are important components of the maternal systemic vasculature because they play a critical role in regulating blood pressure during pregnancy by contributing to total peripheral vascular resistance [19]. Because omental arteries are easily accessible and safely harvested during a variety of surgeries in women, they offer a valuable opportunity to study gestation-related vascular reactivity to a variety of agents. In addition, adequate lengths of omental artery can be easily obtained without significant dissection,

[1]This work was supported by National Institutes of Health grants HL102866 and HL58144 to C.Y. Presented in part as a poster presentation at the SRI Annual Meeting, March 25-28, 2015, San Francisco, California; abstract no. T-213.
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Received: 22 September 2015.
First decision: 14 October 2015.
Accepted: 20 October 2015.

eISSN: 1529-7268 http://www.biolreprod.org
ISSN: 0006-3363

ensuring a stable and replicable human vascular tissue model. It is unknown whether the CALCB family of peptides regulates omental artery relaxations and whether these effects are elevated during pregnancy in women to facilitate vascular adaptions. Further, the mechanisms involved in the vasodilatory responses of human omental artery to the CALCB family of peptides are also unknown. Evidence from both an animal model and from clinical research has demonstrated that dysfunction in CALCB activity may contribute to the increase in blood pressure in preeclampsia [20–23]. Therefore, in this study we examined the role of the CALCB family of peptides in regulating omental artery relaxation responses in nonpregnant and pregnant women and assessed the underling mechanisms of relaxation.

MATERIALS AND METHODS

Human Subjects

The protocol for this study was approved by the Baylor College of Medicine Institutional Review Board (Institutional Review Board no. H28527) and was conducted according to Declaration of Helsinki Principles. Patients undergoing elective procedures for benign gynecologic indications, and patients undergoing cesarean delivery were enrolled. All patients gave informed written consent. Nonpregnant patients ($n = 15$) were premenopausal and were undergoing hysterectomy for uterine leiomyoma or dysfunctional uterine bleeding unresponsive to prior medical management (e.g., oral contraceptives and/or medroxyprogesterone). As per standard clinical practice at our institution, all nonpregnant patients received cefazolin preoperatively. Any specimens collected from women with a history of peripheral vascular disease, diabetes mellitus requiring insulin therapy, or uncontrolled hypertension were excluded. Pregnant women underwent cesarean delivery ($n = 15$) for the following obstetric indications: scheduled repeat cesarean delivery, malpresentation, and placenta previa. All pregnant patients also received prophylactic antibiotics (most commonly cefazolin 2 or 3 g, based on body mass index, or clindamycin and gentamicin for patients with cephalosporin allergy). Pregnant patients were excluded from participating in the study if they had any of the following: diabetes, fetal anomalies, multifetal pregnancy, hypertension, preeclampsia, or clinical evidence of maternal or fetal infection.

Omental Artery Preparation and Isometric Tension Recording

Omental fat biopsies containing a representative vessel were immediately placed in cold oxygenated physiological salt solution (PSS), consisting of 118.2 mM NaCl, 24.8 mM $NaHCO_3$, 4.6 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, and 10.0 mM dextrose. A small artery (200-μm normalized internal diameter) was cleaned, cut, and dissected into 2-mm rings, which were mounted on a Multi Wire Myograph System (Model 620M; Danish Myo Technology) for isometric tension recording, as previously described [24, 25]. The myograph bath contained PSS at 37°C and was bubbled with 5% CO_2 in O_2. To induce contraction, arterial rings were held at optimal tension of 2.5 mN and then exposed to potassium chloride (80 mM). After washing and re-equilibration in PSS, the arterial rings were precontracted with thromboxane agonist U46619 (1 μM). Cumulative dose-response curves were constructed for CALCB, ADM, and ADM2 (0.1–100 nM). The relaxation responses were calculated as percent inhibition of the U46619 initially induced contraction, and the maximum possible effects for the reagent (Emax) were calculated accordingly.

Isolation of Total RNA and RT

Omental arteries were quickly isolated from fat, the arteries were snap-frozen in liquid nitrogen, and total RNA was isolated using TRIzol (Life Technologies). RNA extraction was followed by RNA cleanup (Qiagen) treatment to remove DNA contamination. The quality and quantity of the RNA were assessed at absorbance 260 and 280 nm, and all samples showed absorbency ratios ranging between 1.8 and 2.0. For RT, 1 μg of total RNA was mixed with 3.0 nmol of random primer (Invitrogen), 200 μM dinucleotide triphosphate solution (Sigma), and 10 units of AMV reverse transcriptase (Promega) in the presence of 5 units of RNase inhibitor (Invitrogen), and was placed in a thermal cycler for one cycle at 28°C for 15 min, 42°C for 30 min, 99°C for 5 min, and 4°C for 5 min.

Quantitative Real-Time PCR

Real-time quantitative RT-PCR was performed by using TaqMan probes for CALCRL, RAMP1, RAMP2, and RAMP3, produced by Life Technologies. Amplification of housekeeping GAPDH (forward primer: 5′-GGTCTCCTCTGACTTCAACA-3′, and reverse primer: 5′-AGC CAAATTCGTTGTCATAC-3′) served as an endogenous control to standardize the amount of sample RNA added to a reaction. The PCR conditions for TaqMan Gene Expression Assay were 2 min at 50°C and 10 min at 95°C for 1 cycle, then 15 sec at 95°C and 1 min at 60°C for 40 cycles. All experiments were performed in triplicate. Negative controls were performed by replacing RNA templates with nuclease-free water. For no-RT control, nuclease-free water was used in place of the reverse transcriptase. Results were calculated using the $2^{-\Delta\Delta CT}$ method and expressed in folds increase/decrease of the gene of interest.

Immunofluorescent Imaging Analysis

Omental arteries were carefully dissected to exclude any fat tissue and then embedded in optimal cutting temperature medium and flash frozen. Tissues were cut at 5- to 7-μm thickness and mounted on gelatin-coated slides, after which the sections were fixed with a mixture of methanol and acetone (1:1) and washed in PBS-T solution. After blocking with PBS supplemented with 10% donkey serum, the first primary antibody, goat polyclonal CALCRL antibody (Santa Cruz Biotechnology Inc.), rabbit RAMPs 1, 2, and 3 polyclonal antibodies (raised against the peptides corresponding to the intracellular C-terminal domain of RAMPs 1, 2, 3 and affinity purified [26]) were applied at 1:100 dilution, followed by donkey anti-goat AlexaFluor 594/anti-rabbit immunoglobulin G (IgG) TRI-TC (Life Technologies). For double immunostaining, the slides stained with first primary antibody were thoroughly rinsed with PBS-T, followed by the application of the second primary (monoclonal) antibody, mouse anti-human CD31 antibody (BD Biosciences) at 1:150 dilution for 60 min. Donkey anti-mouse AF488 was used for detection of the second primary antibody. Absence of antibody, preimmune serum, or mouse IgG Ready to Use (Dako) was used as negative control. Slides were then counterstained with 4′,6-diamidino-2-phenylindole (Vector Laboratories Inc.), mounted with Mounting-medium (Dako), and viewed under an Olympus BX51 microscope. The images were recorded by using a DP70 Digital Camera (Olympus Optical Co. Ltd.).

Mechanisms of CALCB Peptide-Induced Omental Artery Relaxation

The involvement of possible intracellular messengers, and ion channels in CALCB-, ADM-, and ADM2-induced vascular relaxation were investigated using selective inhibitors. Omental arteries precontracted with U46619 (1 μM) were first exposed to varying doses of CALCB, ADM, and ADM2 (0.1–100 nM). After washing with PSS, the omental arterial rings were incubated for 30 min in PSS with either the inhibitors of NO synthase (*N*-nitro-L-arginine methyl ester [L-NAME]; 100 μM), adenylyl cyclase (SQ22536; 10 μM), voltage-sensitive potassium channel (tetrabutylammonium [TBA; 1 mM] and 4-aminopyrodin [0.1 mM]), ATP-sensitive potassium channel (glibenclamide; 10 μM), Ca^{2+}-activated potassium channel (charybdotoxin [CHX; 0.1 μM], apamin [10 μM], and tetraethylammonium [TEA; 1 mM]), and the cyclooxygenase inhibitor indomethacin (Indo; 10 μM). After incubation, relaxation responses to cumulative doses of CALCB, ADM, and ADM2 were repeated in U46619 precontracted arterial segments. Relaxation responses were calculated as percent inhibition of the U46619 initially induced contraction, and the maximum possible effects for the reagent (Emax) were calculated and compared.

Drugs

CALCB, ADM, and ADM2 were purchased from Phoenix Pharmaceutical Inc. U46619 and SQ22536 were purchased from Cayman Chemical Company. All other reagents, including bradykinin, L-NAME, Indo, TEA, TBA, CHX, apamin, glibenclamide, and 4-aminopyridine, were purchased from Sigma Chemical.

Statistical Analysis

All data were presented as mean ± SEM. Relaxation responses to the CALCB family of peptides were expressed as percent relaxation of the initial U46619-induced contraction. Vasodilator concentration response curves were fitted to a log-logistic sigmoid relation, and Emax (maximal relaxation effect)

was calculated by using GraphPad Prism. Repeated-measures ANOVA (treatment and time as factors) with a Bonferroni post hoc test were used for comparisons of dose-response curves between groups. The mRNA expressions were compared between control and treatment groups using unpaired Student *t*-test. Statistical significance was defined as $P < 0.05$.

RESULTS

Influence of Pregnancy on the Vasodilatory Responses of Omental Artery to CALCB, ADM, and ADM2

CALCB, ADM, and ADM2 produced dose-dependent relaxations in U46619 precontracted omental artery obtained from both nonpregnant and pregnant women (Fig. 1). The Emax values of omental arteries to CALCB and ADM2 were significantly enhanced in pregnant compared with nonpregnant women (CALCB: 59.46 ± 2.0 in pregnant vs. 35.96 ± 4.1 in nonpregnant, $P = 0.0002$; ADM2: 48 ± 2.69 in pregnant vs. 37.66 ± 3.71 in nonpregnant, $P = 0.0408$). No significant differences in responses to ADM were observed between nonpregnant and pregnant groups, suggesting an increased sensitivity of omental artery to CALCB and ADM2 during pregnancy.

Messenger RNA Expressions for CALCRL and RAMPs in Omental Artery

To examine whether the CALCB family peptide receptor components CALCRL and RAMPs 1, 2, and 3 are expressed in human omental artery, we analyzed mRNA levels by real-time PCR using the specific primers. As shown in Figure 2A, mRNAs encoding *CALCRL* and *RAMP1*, *RAMP2*, and *RAMP3* were expressed in the human omental artery. Relative mRNA expressions of *CALCRL* and *RAMP1*, *RAMP2*, and *RAMP3* in the omental artery were significantly greater in pregnant women compared with nonpregnant women, suggesting elevated receptor expression during pregnancy for all three peptides.

Cellular Localization of CALCRL and RAMPs in Omental Artery

Using immunofluorescent staining, we found that CALCRL and RAMP1, RAMP2, and RAMP3 were present in the omental artery (Fig. 2B), with staining mainly localized to endothelial cells and smooth muscle cells of the vessels, implying that CALCB, ADM, and ADM2 could exert their relaxation response through their receptors in these arteries. The density of staining was greater in the omental artery from pregnant compared with nonpregnant women, suggesting upregulation in pregnancy. Control sections without primary antibody showed no specific staining in the omental artery segments (data not shown).

Underlying Mechanisms of CALCB-Induced Omental Artery Relaxation

To assess mechanisms mediating CALCB-induced dilation, we examined the dose-dependent vasodilatory responses of omental artery to CALCB in the presence of specific inhibitors of relaxation using omental artery from pregnant women. The inhibitor of NO synthase, L-NAME (100 μM; Fig. 3A), and the blocker of voltage-activated potassium channels, 4-aminopyrodin (0.1 mM; Fig. 3D), produced a parallel shift upward of the concentration-response curves to CALCB in a dose-dependent manner, with a significantly reduced Emax for the treatments with L-NAME (36.54 ± 11.59 vs. 61.95 ± 3.91 in control, $P = 0.0328$) and 4-aminopyrodine (34.90 ± 24.38 vs. 61.95 ± 3.91 in control, $P = 0.0376$). However, TEA, SQ22536, Indo, TBA, apamin, glibenclamide, and CHX did not significantly impact CALCB-induced omental artery relaxation.

Underlying Mechanisms of ADM-Induced Omental Artery Relaxation

We found that the concentration-dependent vasodilatory response of omental artery from pregnant women to ADM was shifted upward in the presence of adenylyl cyclase inhibitor SQ22536 (10 μM; Fig. 4A), voltage-sensitive potassium channel blocker TBA (1 mM; Fig. 4C), and Ca^{2+}-activated potassium channel blocker CHX (0.1 μM; Fig. 4D), with significantly reduced Emax values for SQ22536 (28.1 ± 8.94 vs. 57.09 ± 3.56 in control, $P = 0.0016$), TBA (33.98 ± 10.71 vs. 57.09 ± 3.56 in control, $P = 0.0153$), and CHX (33.93 ± 15.42 vs. 57.09 ± 3.56 in control, $P = 0.0484$). However, L-NAME, TEA, Indo, glibenclamide, apamin, and 4-aminopyrodine did not significantly influence ADM-induced relaxation of these vessels.

Underlying Mechanisms of ADM2-Induced Omental Artery Relaxation

The adenylyl cyclase inhibitor SQ22536 (10 μM; Fig. 5A), cyclooxygenase inhibitor Indo (10 μM; Fig. 5B), and Ca^{2+}-activated potassium channel blocker CHX (0.1 μM; Fig. 5D), produced a parallel shift upward of the concentration-response curves to ADM2, with significantly reduced Emax values for SQ22536 (41.09 ± 4.58 vs. 63.89 ± 3.85 in control, $P = 0.001$), Indo (82.72 ± 5.17 vs. 63.89 ± 3.85 in control, $P = 0.0229$), and CHX (38.79 ± 12.72 vs. 63.89 ± 3.85 in control, $P = 0.0208$). However, L-NAME, TEA, glibenclamide, TBA, apamin, and 4-aminopyrodin failed to influence ADM2-induced omental artery relaxation.

DISCUSSION

The present study provides the first evidence that CALCB, ADM, and ADM2 dose-dependently relax omental artery from nonpregnant and pregnant women. The vasodilatory response of omental artery to CALCB and ADM2 is significantly enhanced in pregnant compared with nonpregnant women. Further, their receptor components, CALCRL and RAMPs 1, 2, and 3, are abundantly expressed in endothelial and smooth muscle cells of omental artery, and their expression is substantially increased in the pregnant compared with the nonpregnant state. Thus, it appears that the CALCB family of peptides relax human omental artery and that the vasodilatory responses to CALCB and ADM2, but not ADM, are increased with pregnancy and may play a role in vascular adaptation during pregnancy. Moreover, the relaxing actions of the CALCB family of peptides are mediated through diverse mechanisms, including NO, EDHFs, prostaglandins, and a variety of potassium channels.

In most systemic vascular beds, large arteries and veins contribute little to the total vascular resistance of the circulation and therefore are not critical for the control of blood pressure. In contrast, resistance arteries, such as the mesenteric and omental arteries, substantially contribute to blood pressure regulation [1, 4, 19]. We have previously shown that systemic administration of all three peptides, CALCB, ADM, and ADM2, in rats produces dose-dependent decreases in blood pressure in vivo, and that these responses are greater during pregnancy [15–17]. In addition, CALCRL and all three

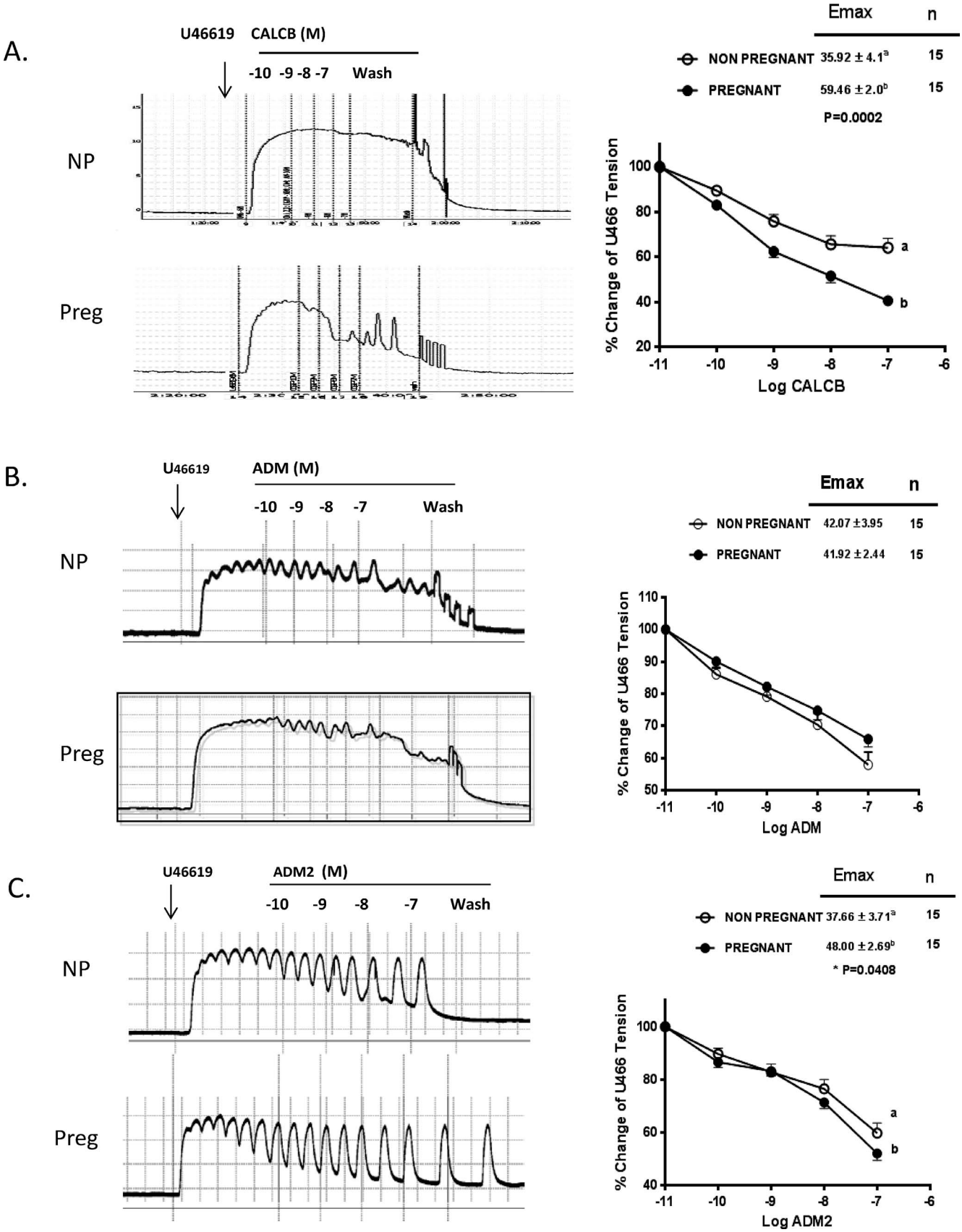

FIG. 1. Vasodilatory responses of omental arteries to CALCB, ADM, and ADM2. **A**) Representative traces showing vascular relaxation induced by CALCB in human omental artery from nonpregnant and pregnant women, and dose-response curves confirming increased relaxation to CALCB in omental artery from pregnant women (n = 15) compared with nonpregnant patients (n = 15). **B**) Representative traces showing vascular relaxation induced by ADM in human omental artery from nonpregnant and pregnant tissue, and dose-response curves indicating no significant difference in response to ADM between the two groups. **C**) Representative traces showing vascular relaxation induced by ADM2 in human omental artery, and dose-response curves confirming

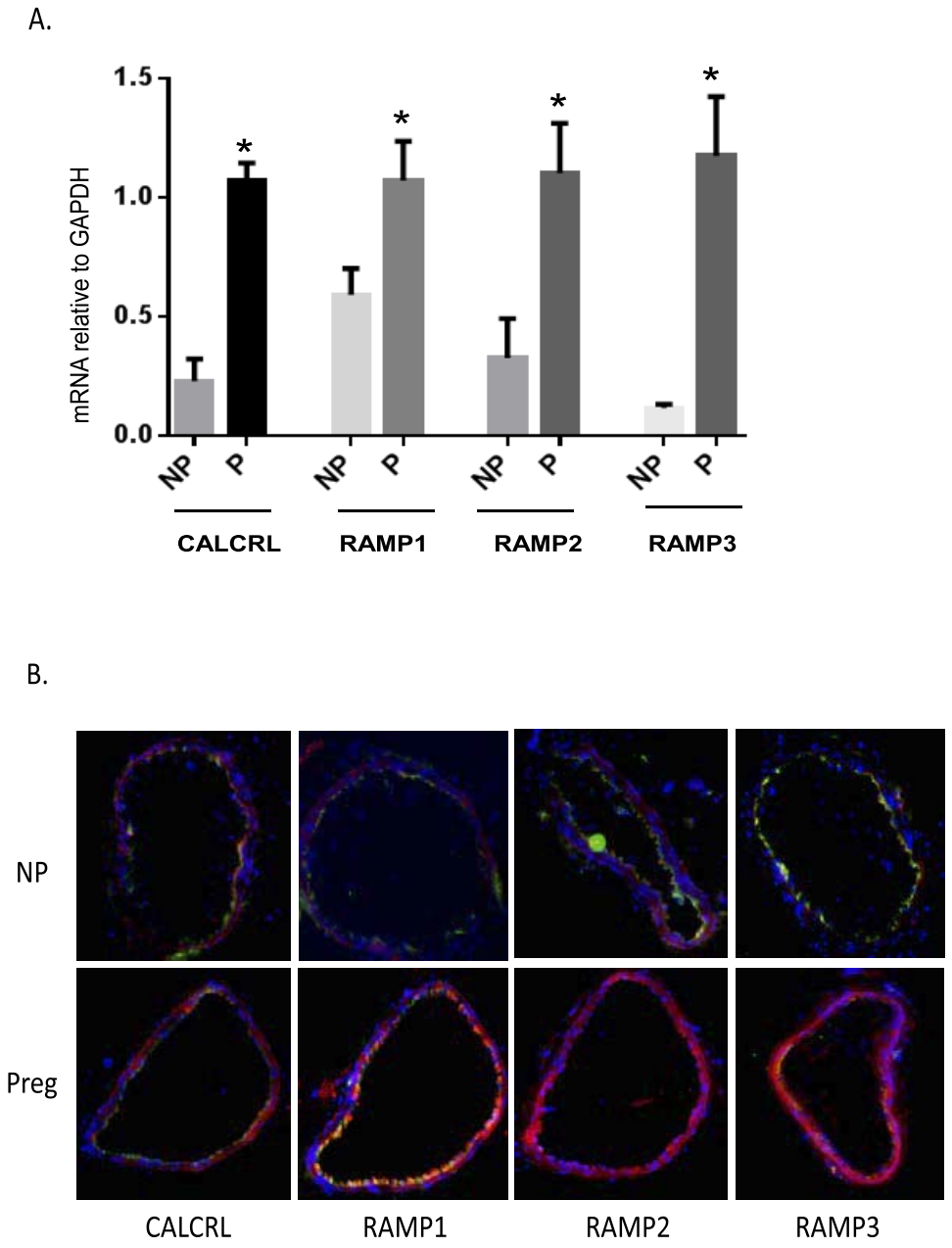

FIG. 2. The mRNA and protein expressions of CALCRL, RAMP1, RAMP2, and RAMP3 in omental arteries. **A**) The mRNA expression of CALCRL and RAMPs 1, 2, and 3 in omental arteries from nonpregnant (NP; n = 15) and pregnant (P; n = 15) women. CALCRL, RAMP1, RAMP2, and RAMP3 mRNA expressions were normalized to GAPDH mRNA expression. Data are displayed as mean ± SEM. *$P < 0.05$ versus control. **B**) Immunofluorescent localization of CALCRL (red) and RAMPs 1–3 (red) in sections of the omental artery from nonpregnant (NP) and pregnant (Preg) women. Endothelial cells (green) were identified by antibody to CD31. CALCRL, RAMP1, RAMP2, and RAMP3 are abundantly expressed on endothelial and smooth muscle cells of the arteries from pregnant women. Original magnification ×200.

RAMPs are expressed in rat mesenteric and uterine arteries, and their levels are elevated during pregnancy [15, 17, 27–29], which is consistent with an enhanced vasodilatory response of these vessels to the CALCB family of peptides [4]. Similar to our studies in rats, the current study demonstrated that the CALCRL and RAMPs are expressed in human omental artery, that their expression is greater in pregnant versus nonpregnant women, and that they are primarily localized to endothelial and smooth muscle cells (Fig. 2). In addition, all three peptides induce relaxation of omental artery in a dose-dependent manner (Fig. 1), and this response is greater in pregnant compared with nonpregnant women for CALCB and ADM2, but not for ADM. Based on these findings, we suggest that CALCB and ADM2 may play a role in decreasing peripheral vascular resistance in the human during pregnancy. The lack of an enhanced effect of ADM on human omental artery during pregnancy may relate to changes in postreceptor signaling and warrants further investigation.

increased relaxation to ADM2 in omental artery from pregnant (n = 15) compared with nonpregnant (n = 15) women. Results are shown as mean ± SEM. Different letters indicate significant differences ($P < 0.05$) between groups. NP, nonpregnant; Preg, pregnant.

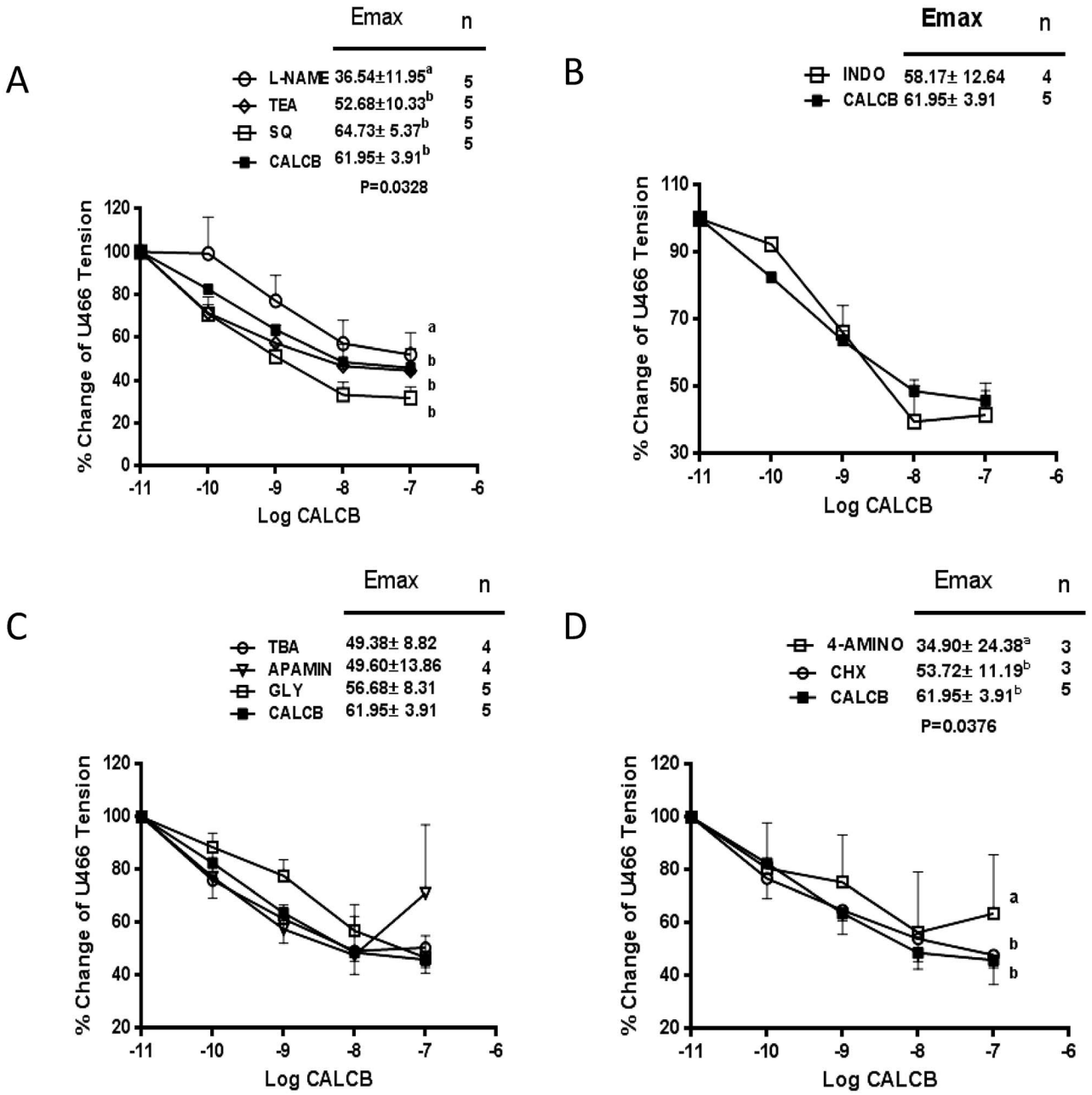

FIG. 3. Underlying mechanisms of CALCB-induced omental artery relaxation. The effects of (**A**) the NO synthase inhibitor L-NAME (0.1 mM), the Ca^{2+}-dependent potassium channel blocker TEA (10 μM), and the adenylyl cyclase inhibitor SQ22536 (SQ; 10 μM); (**B**) the cyclooxygenase inhibitor indomethacin (INDO; 10 μM); (**C**) the nonselective potassium channel blocker TBA (1 mM), the Ca^{2+}-activated K^+ channel blocker apamin (10 μM), and the ATP-sensitive potassium channel blocker glibenclamide (GLY; 10 μM); and (**D**) the voltage-activated potassium channel blockers 4-aminopyrodin (4-AMINO; 0.1 mM) and CHX (0.1 μM) on vasodilatory responses to CALCB in omental artery from pregnant women. Results are shown as mean ± SEM. Different letters indicate $P < 0.05$ between groups.

The mechanisms through which these peptides induce human omental artery relaxation are currently unknown. Several studies of resistance vessels in animals have shown that these peptides act through a variety of pathways. These include activation of endothelial NO synthase [6], generation of cAMP [30], and modulation of different potassium channels [4, 31]. In addition, these peptides appear to exert their relaxation responses in a vessel-specific manner [4]. In the current study, CALCB-induced omental artery relaxation was inhibited by L-NAME and 4-aminopyrodin (Fig. 3), suggesting the involvement of the NO pathway in endothelial cells and voltage-activated potassium channels in the vascular smooth muscle. However, in rat mesenteric and uterine arteries, CALCB-induced relaxations appear to be NO independent but involve cAMP production and ATP-sensitive potassium channel activation [27, 32]. Moreover, CALCB-induced relaxation was associated with a significant increase in both cGMP and cAMP production in rat aorta [33] and human internal mammary artery [34], supporting the notion that the mechanisms involved in CALCB-induced relaxation are vessel specific. Although the present study did not directly address the role of the endothelium in CALCB-induced omental artery vasodilation, our data showing that L-NAME inhibited CALCB actions suggest that NO

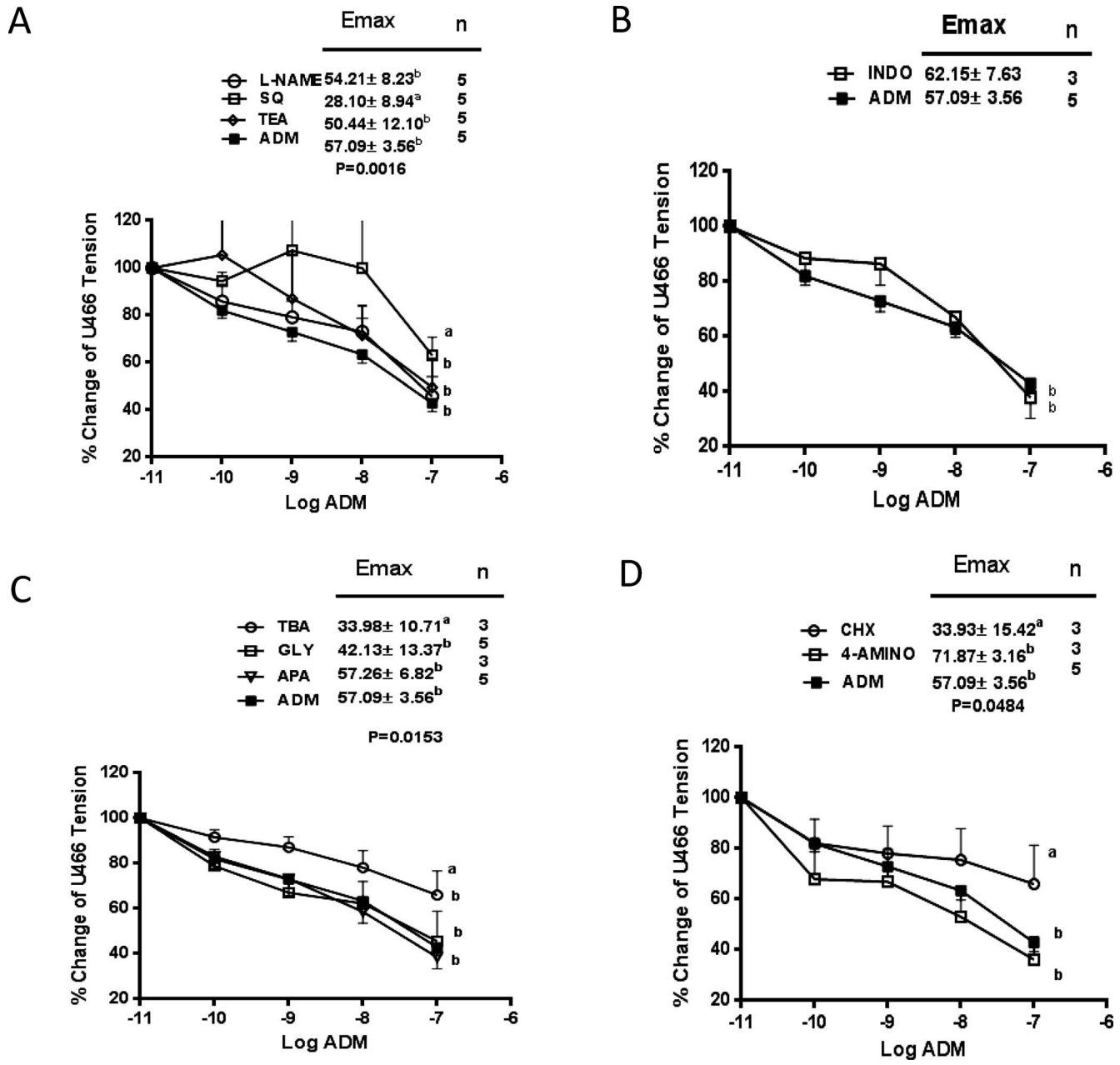

FIG. 4. Underlying mechanisms of ADM-induced omental artery relaxation. The effects of (**A**) L-NAME (0.1 mM), SQ22536 (SQ; 10 μM), and TEA (10 μM); (**B**) indomethacin (INDO; 10 μM); (**C**) TBA (1 mM), glibenclamide (GLY; 10 μM), and apamin (APA; 10 μM); and (**D**) CHX (0.1 μM) and 4-aminopyradin (4-AMINO; 0.1 mM) on vasodilatory responses to ADM in omental artery from pregnant women. Results are shown as mean ± SEM. Different letters on indicate $P < 0.05$ between groups.

synthesis contributes to CALCB-induced relaxation in human omental artery.

We have previously shown that ADM-induced hypotensive effects and the relaxation of both mesenteric and uterine artery to ADM are greater during pregnancy than in the nonpregnant state in rats [17, 29]. ADM has been shown to induce vascular relaxation via both endothelium-dependent [35] and independent [36] pathways, and in the mesenteric arteries both of these pathways appear to mediate ADM actions [17, 24]; in uterine artery, however, the endothelium-dependent pathway is primarily involved [29]. Furthermore, ADM-induced rat mesenteric artery relaxation involved cAMP as well as both ATP-sensitive and Ca^{2+}-activated potassium channels [17, 24]. In the uterine artery ADM-induced relaxation appears to involve cAMP and Ca^{2+}-activated potassium channels [29]. In the present study, the ADM-induced vasorelaxation of omental artery does not appear to involve NO, because L-NAME was ineffective. However, ADM effects were reduced by SQ22536 (Fig. 4), suggesting the involvement of the cAMP pathway. In addition, voltage-sensitive and Ca^{2+}-activated potassium channels appear to be involved, because both TBA and CHX inhibited ADM-induced omental artery relaxation (Fig. 4). This suggests that ADM-induced human omental artery relaxation is predominantly cAMP and potassium channel mediated. However, the vasodilatory response of omental artery to ADM is not increased in pregnant women, suggesting a limited

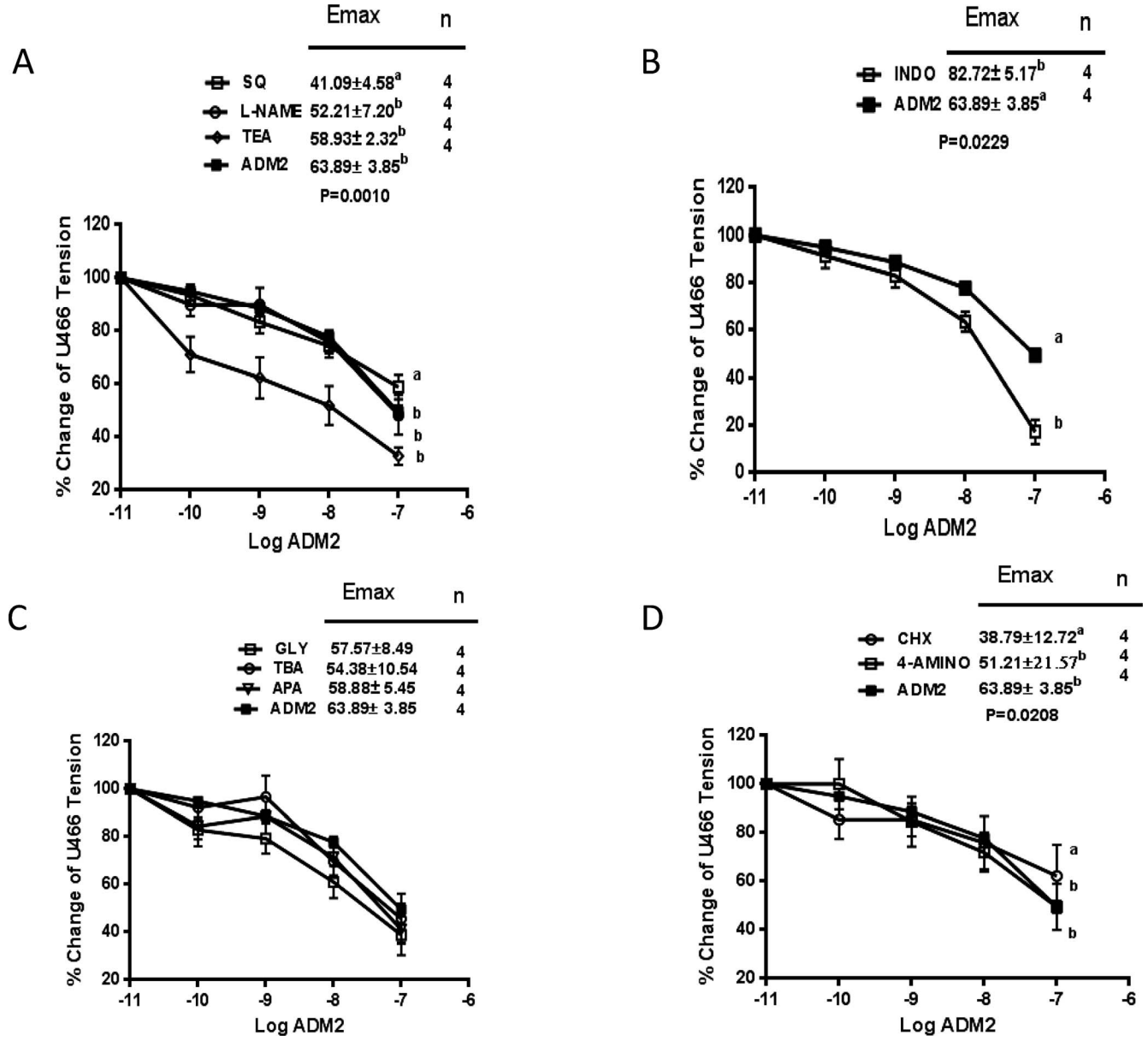

FIG. 5. Underlying mechanisms of ADM2-induced omental artery relaxation. The effects of (**A**) SQ22536 (SQ; 10 μM), L-NAME (0.1 mM), and TEA (10 μM); (**B**) indomethacin (INDO; 10 μM); (**C**) glibenclamide (GLY; 10 μM), TBA (1 mM), and apamin (10 μM); and (**D**) CHX (0.1 μM) and 4-aminopyradin (4-AMINO; 0.1 mM) on vasodilatory responses to ADM2 in omental artery from pregnant women. Results are shown as mean ± SEM. Different letters indicate $P < 0.05$ between groups.

role for ADM in reducing systemic vascular resistance during pregnancy.

Rat mesenteric artery relaxation to ADM2 was also enhanced during pregnancy, and this was associated with increased cAMP and cGMP generation, and it involved Ca^{2+}-activated potassium channels [15]. In the present study human omental artery relaxation to ADM2 was inhibited by the adenylyl cyclase inhibitor SQ22536 and by CHX (Fig. 5), implicating cAMP and Ca^{2+}-activated potassium channels in ADM2-induced human omental artery relaxation. Moreover, unlike CALCB and ADM, ADM2-induced omental artery relaxation was inhibited by the cyclooxygenase blocker indomethacin, suggesting that endothelium-derived PGI_2 may also play a role in ADM2-induced, but not in CALCB- and ADM-induced, omental artery relaxation in pregnancy.

In summary, our study shows that the CALCB family of peptides relax human omental artery and that the vasodilatory responses to CALCB and ADM2 are greater during pregnancy. These peptides, acting through enhanced receptor levels in the omental artery, may contribute to vascular adaptations during pregnancy in women. Moreover, these CALCB family peptides appear to exert their relaxation effects through diverse mechanisms, including NO, EDHF, and prostaglandins, as well as through a variety of potassium channels (Fig. 6). We therefore suggest that the CALCB family of peptides may play a role in enhancing vascular relaxation during pregnancy in the human. It is also possible that disturbances in these peptide-related effects could be involved in the pathogenesis of hypertensive disorders during pregnancy, including preeclampsia and gestational hypertension.

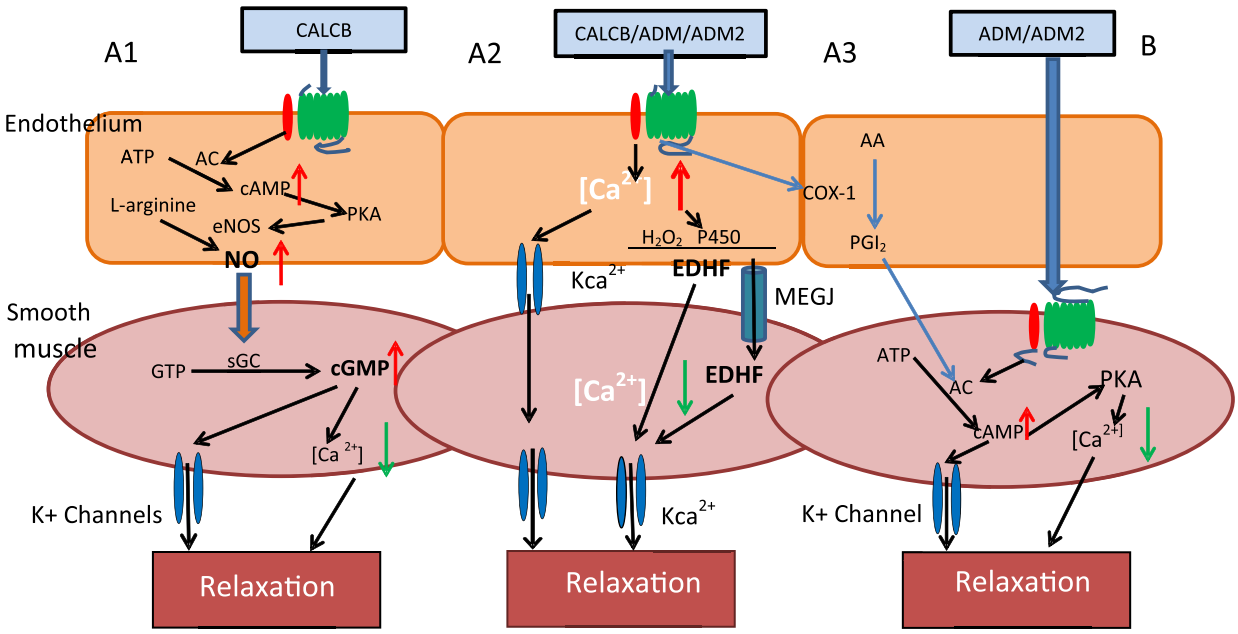

FIG. 6. Signal transduction in CALCB-, ADM-, and ADM2-induced omental artery relaxation. **A)** Endothelium-dependent vasodilatation of human omental artery to CALCB, ADM, and ADM2. **A1**) CALCB, ADM, and ADM2 interact with receptors on endothelial cells and stimulate cAMP accumulation and protein kinase A (PKA) expression, then increase NO production through endothelial nitric oxide synthase (eNOS) activation. Diffusion of NO into adjacent smooth muscle cells increases cGMP generation, leading to smooth muscle relaxation. **A2**) CALCB family peptides bind to their receptors on endothelial cells, leading to the release of intracellular Ca^{2+} and synthesis of EDHF, including cytochrome P-450 (CYP450), K^+, and H_2O_2. EDHF diffuses to the vascular smooth muscle cells, activates KCa channels, and causes endothelium-dependent hyperpolarization, in which myoendothelial gap junctions (MEGJs) may serve as a tie for electrical signal transduction. **A3**) ADM2 binds to its receptors on endothelial cells, activates COX-1, stimulates PGI_2 production, and then relaxes the smooth muscle by increasing cAMP generation. **B**) Endothelium-independent vasodilatation to CALCB: activation of CALCB receptors on smooth muscle cells is coupled to production of cAMP by adenylyl cyclase. The increase in intracellular cAMP stimulates PKA, which opens K^+ channels and activates Ca^{2+} sequestration mechanisms to cause smooth muscle relaxation.

ACKNOWLEDGMENT

The author would like to thank Ms. Uma Yallampalli and Ms. Sandra Dale for their assistance with this work.

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