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IN PREECLAMPSIA ENDOGENOUS CARDIOTONIC STEROIDS INDUCE VASCULAR FIBROSIS AND IMPAIR RELAXATION OF UMBILICAL ARTERIES

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Abstract

Background—Marinobufagenin (MBG), a bufadienolide cardiotonic steroid, induces cardiovascular fibrosis. Because levels of MBG in preeclampsia are increased, and anti-MBG monoclonal antibody reduces blood pressure (BP) in a rat model of preeclampsia, we hypothesized that in preeclampsia, elevated MBG levels would be associated with the development of fibrosis in feto-placental circulation and with impairment of vascular relaxation.

Method—We studied 16 patients with preeclampsia (systolic BP150 +/- 4 mmHg; 28+/-2 years, 37+/-1 weeks gestational age) and 14 gestational age-matched normal pregnant women (systolic BP 112+/-2 mmHg).

Results—Preeclampsia was associated with a rise in plasma and placental levels of MBG. In preeclamptic umbilical arteries, the expression of Fli-1, a transcription factor and a negative regulator of fibrosis, was significantly reduced ($P<0.001$), whereas procollagen-1 expression was increased ($P<0.01$). As compared to control vessels, isolated rings of umbilical arteries from patients with preeclampsia demonstrated unaltered responsiveness to endothelin-1 (EC50=2.2 and 3.2 nmol/l, respectively), but exhibited an impaired response to the relaxant effect of sodium nitroprusside (EC50=1.5 vs. 32.4 nmol/l, $P<.001$) following endothelin-1-induced constriction. Ex-vivo treatment of normal umbilical arteries explants with 1 and 10 nmol/l MBG for 24 h mimicked the effects of preeclampsia, specifically suppressed Fli-1 and increased collagen-1 expression while impairing vasorelaxation.

Conclusion—Our results indicate that in preeclampsia, elevated levels of MBG induce vascular fibrosis via a Fli-1-dependent mechanism which leads to an impairment of vasorelaxation, and suggest that MBG represents a potential target for therapy of this syndrome.

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INTRODUCTION

Altered placental production of vasoactive and angiogenic substances appears to underlie the pathogenesis of hypertension and vascular dysfunction in preeclampsia [1,2]. Endogenous cardiotonic steroids (CTS), i.e., endogenous digitalis-like inhibitors of the Na/K-ATPase are counted among these substances [3]. In fact, elevated plasma levels of CTS were detected in patients with preeclampsia more than 20 years ago [4,5]. In several studies, Digibind, Fab fragments of ovine digoxin antibody which interacts with endogenous CTS, was demonstrated to reduce blood pressure in preeclamptic patients [6-8]. Our laboratory demonstrated that in preeclampsia plasma levels of a bufadienolide CTS, marinobufagenin (MBG), increase, and that ex vivo anti-MBG antibody reverses preeclampsia-induced inhibition of erythrocyte Na/K-ATPase, a target enzyme for CTS [9-11]. In pregnant NaCl-supplemented rats increased MBG levels accompanied development of symptoms of preeclampsia including elevation of arterial pressure, proteinuria and reduction of fetal weight and size [11,12]. In this model, in vivo immunoneutralization of MBG with poly- and monoclonal antibodies produced antihypertensive effect associated with the increase in vascular Na/K-ATPase activity [11,12].

Recently, we demonstrated that nanomolar concentrations of MBG stimulate synthesis of collagen and induce fibrosis in cardiovascular tissues and in the kidney [13,14]. One of the mechanisms underlying the pro-fibrotic effect of MBG is PKC-delta-dependent inhibition of Fli-1, a nuclear transcription factor and a negative regulator of collagen synthesis [5]. Considering that, (i) MBG stimulates synthesis of collagen, (ii) that levels of MBG are elevated in preeclampsia, and (iii) that preeclampsia is associated with vascular stiffening [16,17], we hypothesized that MBG-induced Fli-1-dependent synthesis of collagen is implicated in the development of vascular fibrosis and in the impairment of vasorelaxation in preeclampsia. In the present study we examined impact of preeclampsia on the levels of MBG, on expression of Fli-1 and collagen-1 in umbilical arteries, and on vasorelaxant properties of umbilical arteries. Next, ex vivo, in explants of umbilical arteries from subjects with uncomplicated pregnancies, we studied effect of MBG on expression of Fli-1 and collagen-1 and on endothelium-independent vasorelaxation.

METHODS

The protocol for the study was approved by the Research Council of School of Pediatric Medicine, St. Petersburg, Russia, and by the Institutional Review Board of Medstar Research Institute, Washington, DC. Consecutive patients with preeclampsia who were admitted to 11th and 17th Maternity Hospitals (St. Petersburg, Russia) were enrolled in the study. Preeclampsia was diagnosed according to the criteria established by the American College of Obstetrics and Gynecology [18]. This definition includes at least two of the following criteria: a diastolic BP of at least 90 mmHg, a systolic BP of at least 140 mmHg, an increase in the diastolic BP of at least 15 mmHg, or an increase in systolic BP of 30 mmHg on at least two occasions 6 h or more apart; proteinuria defined by at least 300mg protein in a 24-h urine collection or a protein concentration of 1 g or more per liter in two random urine specimens collected 6 h or more apart; and edema defined as a generalized accumulation of fluid of greater than 1þ pitting edema after 12 h of bed rest or a weight gain of 5 pounds or more in 1 week in the setting of pregnancy after the 20th week of gestation. Gestationally age-matched women with uncomplicated pregnancies served as controls. None of the women studied had ever taken digitalis drugs or had a chronic disease known to be associated with increased levels of CTS (specifically, hypertension, renal or hepatic diseases or endocrine dysfunction).

Placentae

Placentae were perfused with a solution containing (in mmol/l) NaCl 120; KCl 4; $CaCl_2$ 2.5; $MgCl_2$ 2.0; NaH_2PO_4 1.1; $NaHCO_3$ 24; and glucose 5.6 (pH 7.4) until complete removal of blood was accomplished, and tissue was then minced and homogenized. Homogenates were divided into two parts one of which was immediately frozen for determination of angiogenic factors by Western blotting (below), and another used for measurement of MBG. For MBG measurement, the homogenate was extracted with chloroform and dried under a vacuum. The dried extract was sonicated in water (1 : 5 w/v) and applied on a reverse-phase C-18 SepPak 'long body' cartridge, eluted with 80% acetonitrile, and dried in a SpeedVac centrifuge (Savant, Hicksville, New York, USA). Levels of MBG were determined in placental extracts as described below (Immunoassays). Placental homogenates for western blotting were processed with Polytron 20S homogenizer (Kinematica, Switzerland) in 250mmol/l sucrose and 5 mmol/l histidine solution (48C; pH 7.4), and centrifuged at 10 000g for 30 min at 48C. The resultant supernatant was centrifuged (Beckman L8-N, 148 000g, 90 min, 48C), the pellet was re-suspended in a homogenizing medium to a protein concentration of 3 mg/ml and stored in liquid nitrogen. Levels of endoglin and sFlt1 in placental homogenates were determined by Western blotting (described below).

Umbilical arteries

After the delivery umbilical arteries were separated from surrounding tissues and either immediately tested for contractile/relaxant properties (below) and processed for determination of Fli-1, procollagen-1 and collagen-1 levels. Umbilical arteries from women with uncomplicated pregnancies were also treated ex vivo with MBG to mimic effects of preeclampsia. Explants of umbilical arteries from women with uncomplicated pregnancies were placed in Dulbecco's Modified Eagle Medium supplemented with high glucose, glutamine, pyrodoxin hydrochloride, and sodium pyruvate (25 mg/kg gentamicin) (Invitrogen), and were incubated for 24 h at 378C in the presence of MBG (1 and 10 nmol/l) or vehicle (control). Both freshly obtained and MBG/vehicle-treated vascular rings were used in contractile studies and for preparation of homogenates (below). Arterial rings were minced by scissors, processed with Polytron 20S homogenizer (Kinematica, Switzerland) in 250mmol/l sucrose and 5mmol/l histidine solution (48C; pH 7.4), and centrifuged (5000g, 15 min, 4oC). The pellet was homogenized in a glassteflon homogenizer and combined with the supernatant, which was re-spun at 10 000g for 30 min at 48C, and the resultant supernatant centrifuged (Beckman L8-N, 148 000g, 90 min, 48C). The pellet was re-suspended in a homogenizing medium to a protein concentration of 1 mg/ml and stored in liquid nitrogen.

Isolated umbilical artery contractile studies

Endothelium-denuded rings of umbilical arteries (2.5–4.0mm wide) were suspended at a resting tension of 3.0 g in a 15-ml organ bath (Ugo Basile, Italy) and superfused at 37oC with a solution containing in mmol/l: NaCl 130, KCl 4.0, CaCl2 1.8, MgCl2 1.0, NaH2PO4 0.4, NaHCO3 19, and glucose 5.4, and gassed with a mixture of 95% O2 and 5% CO2 (pH 7.45), and isometric contractions were recorded as reported previously [19]. The arterial rings were constricted twice with 80mmol/l KCl, and after complete relaxation, contractile responses to endothelin-1 (0.1–10 nmol/l) were studied. To investigate the impact of preeclampsia and of MBG treatment on the relaxation of umbilical arteries, we studied the effect of increasing concentrations of sodium nitroprusside (1 nmol/l–1umol/l) following constriction of vascular rings with 100 nmol/l endothelin-1. The force of contractions was expressed as the percentage of vasoconstrictor response to 80 mmol/l KCl. The percentage relaxation was calculated relative to the plateau of contractile force that was achieved in response to 100 nmol/l endothelin-1.

Western blotting

Levels of two preeclampsia markers endoglin-1 (mouse monoclonal antibody MAB0702, Santa-Cruz Biotechnology; 1 : 500) and sFlt1 (rat monoclonal antibody 2Q1707, Santa-Cruz Biotechnology; 1 : 250) were determined in placental homogenates. In membranes from umbilical arteries we determined levels of Fli-1 (rabbit polyclonal antibody; Santa-Cruz Biotechnology; 1 : 500), procollagen-1 (goat polyclonal antibody; Santa Cruz Biotechnology; 1 : 200) and collagen-1 (goat polyclonal antibody, Southern Biotechnology; 1 : 200). Solubilized proteins were separated by 10% Tris-Glycine polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane and were visualized using above specific antibodies followed by incubation with peroxidase-conjugated antimouse or antirabbit antiserum (Amersham Corp., 1 : 1000), or antigoat antiserum(Santa Cruz Biotechnology, 1 : 1000). Bands were visualized by 1–15 min exposure of nitrocellulosemembrane on Kodak SAR5 film.Tonormalize levels of Fli-1, procollagen-1 and collagen-1 against levels of glyceraldehydes-3-phosphate dehydrogenase (GAPDH), membranes were stripped and re-probed with a rabbit monoclonal antibody (mAb) against human GAPDH (Cell Signaling Technology, Inc. Danvers, MA).

Erythrocyte Na/K-ATPase activity

The activity of erythrocyte Na/K-ATPase of patients with preeclampsia and of women with uncomplicated pregnancy was determined in the presence and absence of anti-MBG 3E9 mAb as reported previously [20]. The concentration of mAb used (0.85mg/ml) was that which ex vivo reversed the MBG-induced 75% inhibition of rat kidney Na/K-ATPase and in vivo reduced BP in hypertensive Dahl-S rats as also reported previously [11].

Immunoassays

Marinobufagenin was measured using a fluoroimmunoassay [dissociation-enhanced FluoroImmunoAssay (DELFIA)] based on a murine anti-MBG 4G4 mAb recently described in detail [11]. This assay is based on competition between immobilized antigen (MBGglycoside-thyroglobulin) and MBG, endogenous CTS, or other cross-reactants within the sample for a limited number of binding sites on an anti-MBG mAbs. Secondary goat antimouse antibody labeled with europium was obtained from Perkin-Elmer (Waltham, Massachusetts, USA). The cross-reactivity of 4G4 mAb is (%): MBG – 100; marinobufotoxin – 43; cinobufotalin – 40; telocinobufagin – 14; resibufagenin – 0.5; bufalin – 0.08; cinobufagin – 0.07; digoxin – 0.03; ouabain – 0.005; ouabagenin – 0.001; digoxigenin – 0.004; proscillaridin A, digitoxin, aldosterone, progesterone, prednisone, corticosterone, and thyroglobulin – less than 0.001.

Statistical analyses

Data are presented as means_SEM. Statistical analyses utilized two-tailed t-test or one-way ANOVA followed by a post-hoc t-test utilizing Newman–Keuls correction for multiple comparisons. A two-sided P value of less than 0.05 was considered to be statistically significant (Graph- Pad Instat and GraphPad Prism, GraphPad Software Inc., San Diego, California, USA).

RESULTS

Sixteen patients with preeclampsia (age, 28 ± 2 years; gestational age, 37 ± 1 weeks, urinary protein excretion, 2.12 ± 0.46 g/24 hours) and 14 normotensive pregnant subjects (age, 26 ± 1 years; gestational age, 39 ± 1 weeks) were enrolled in the study. As illustrated in Figure 1, patients with preeclampsia demonstrated an increase in systolic and diastolic blood pressures (Figure 1A,B) and was accompanied by elevated levels of MBG in plasma and placentae

(Figure 1C,D). In the patients with preeclampsia, elevated levels of MBG were associated with a substantial inhibition of Na/K-ATPase in the erythrocytes, as compared to those in subjects with uncomplicated pregnancies, and pretreatment of preeclamptic erythrocytes with 3E9 anti-MBG mAb restored Na/K-ATPase activity (Figure 1E). Levels of two angiogenic factors and markers of preeclampsia, endoglin-1 and sFlt1, were significantly elevated in the preeclamptic placentae as compared to those in the placentae from subjects with uncomplicated pregnancies (Figure 1F,G).

As presented in Figure 2A, development of preeclampsia was associated with a dramatic reduction in the expression of Fli-1 in the umbilical arteries. Conversely, levels of procollagen-1 and collagen-1 in the membranes from umbilical arteries from patients with preeclampsia were elevated two-fold, as compared to those in umbilical arteries obtained from subjects with uncomplicated pregnancies (Figure 2B,C).

Data on ex vivo functional studies with isolated rings of umbilical arteries are summarized in Figure 2D,E. Vascular rings obtained from patients with preeclampsia and from normotensive pregnant subjects exhibited similar sensitivity to vasoconstrictor effect of endothelin-1 (Figure 2D). Thus, the EC_{50} values for the vasoconstrictor effect of endothelin-1 in normal and preeclamptic arteries were 2.2±0.7 nmol/L and 3.2±1.1 nmol/L, respectively ($P>0.05$). Figure 2E demonstrates results of experiment in which we compared vasorelaxant activity of sodium nitroprusside in the rings of normal and preeclamptic umbilical arteries following development of contracture induced by a submaximal concentration of endothelin-1, 100 nmol/L. As presented in Figure 2E, development of preeclampsia was associated with a marked reduction in the sensitivity of vascular rings to vasorelaxant effect of sodium nitroprusside. The EC_{50} values for relaxant effect of sodium nitroprusside in normal and preeclamptic umbilical arteries were 1.5±0.6 nmol/L and 32.4±14.1 nmol/L, respectively ($P<0.01$).

Next, we studied whether ex vivo incubation of explants of umbilical arteries from subjects with non-complicated pregnancies in the presence of nanomolar concentrations of MBG would mimic effects of preeclampsia. As presented in Figure 3A,B, 24 hour incubation of umbilical artery rings in the presence of 1 nmol/L and 10 nmol/L MBG resulted in a concentration-dependent reduction in the levels of Fli-1 and a concomitant increase in the levels of collagen-1. Umbilical artery rings pretreated with MBG and with vehicle exhibited similar sensitivity to submaximal (100 nmol/L) concentration of endothelin-1 (Figure 3C). In umbilical artery rings pretreated with vehicle and precontracted with 100 nmol/L endothelin-1, EC_{50} for vasorelaxant effect of sodium nitroprusside was 8.8±3.4 nmol/L. Pretreatment with 1 nmol/L MBG caused a decrease in the sensitivity of precontracted umbilical artery rings to sodium nitroprusside which did not reach statistical significance (EC_{50} = 31±17 nmol/L, $P=0.05$) whereas pretreatment with 10 nmo/L MBG markedly impaired the responsiveness of vascular rings to the relaxant effect of sodium nitroprusside (EC_{50} = 73±31 nmol/L, $p<0.01$)(Figure 3D).

DISCUSSION

The main observation of our study is that elevated levels of MBG in plasma and the placenta of patients with preeclampsia are associated with impaired relaxation of umbilical arteries, and with a dramatic reduction in the expression of a negative regulator of collagen synthesis, the transcription factor Fli-1 along with a concomitant increase in the expression of procollagen-1 and collagen-1 in the umbilical arteries. Accordingly, ex-vivo 24-h incubation of umbilical arteries explants from women with uncomplicated pregnancies in the presence of MBG mimics the effects of preeclampsia, resulting in reductions in Fli-1, increases in collagen-1, and in the impairment of vasorelaxation.

In the present study, elevation in the levels of procollagen-1 and collagen-1 in the umbilical arteries, that is, development of fibrosis, was associated with marked impairment of endothelium-independent vasorelaxation in the presence of unaltered responsiveness to endothelin-1 ex vivo. Our data agree with a previous observation of increased collagen content in preeclamptic umbilical arteries [21], and with the concept that vascular stiffness plays an essential role in the development of vascular dysfunction in preeclampsia [16,17,22–24]. One would expect that stiffness resulting from elevated vascular levels of collagen would be associated with a decrease in the ability of the vessel to both contract and to relax. In the present experiment, however, responsiveness of preeclamptic and MBG-treated rings of umbilical arteries to the constrictor effect of endothelin-1 was unaltered in the presence of reduced responsiveness to sodium nitroprusside and elevated levels of collagen-1. One explanation of this phenomenon could be that, in the present study, reduction in the sensitivity of umbilical arteries to endothelin-1 due to development of vascular fibrosis might have been counterbalanced by an MBG-induced increase in the sensitivity of vascular smooth muscle cells to endothelin-1. For example, previously, chronic administration of another Na/K-ATPase inhibitor, ouabain, to rats was shown to produce an increase in the sensitivity of thoracic aortae to constrictor action of endothelin-1 [25]. Interestingly, activation of phospholipase C and PKC are the common links between MBG-induced profibrotic signaling [15] and mechanisms underlying vascular effects of endothelin-1 [26].

Previously we demonstrated that elevations in plasma MBG levels accompanied by elevations in arterial pressure occur in patients with preeclampsia as well as in an experimental model for preeclampsia, namely pregnant rats given NaCl supplementation [9–12]. In these pregnant hypertensive rats, the in-vivo administration of anti-MBG monoclonal antibody lowered BP and produced concomitant restoration of sodium pump activity in aorta proving that in this model MBG was responsible for Na/K-ATPase inhibition [11,12]. In agreement with those earlier experimental results, elevation in plasma and placental levels of MBG in patients with preeclampsia in the present study were associated with a substantial inhibition of its target enzyme, Na/K-ATPase, in erythrocytes, and anti-MBG mAb, ex vivo, reversed this preeclampsia-induced Na/K-ATPase inhibition. In the present study, we demonstrate that MBG could also contribute to pathogenesis of preeclampsia by causing impairment of endothelium-dependent vasorelaxation manifested by a marked reduction in the sensitivity of umbilical arteries to the relaxant effect of sodium nitroprusside. The placental levels of MBG in the present study were elevated in preeclampsia and the range of this increase was comparable to the increases in placental expression of two angiogenic factors, endoglin-1 and sFlt1, which have also been implicated in pathogenesis of preeclampsia [27].

The nature of the vascular dysfunction in preeclampsia is complex; most workers in this field agree that it involves vasospasm as well as the impairment of vasorelaxation in both maternal and fetal vascular beds [1,2,22–24,28]. Our present results are consistent with previous data demonstrating attenuated vasorelaxation to sodium nitroprusside and papaverine in preeclamptic chorionic placental arteries in preeclampsia as compared to those from normal pregnancy [29]. This strongly supports the concept that increased vascular stiffness is implicated in pathogenesis of preeclampsia [16,17]. Because in advancing pregnancy endothelium-dependent mechanisms of vasorelaxation of umbilical artery are reduced [30,31], in the present experiment we did not study the impact of preeclampsia and of MBG treatment on endotheliumdependent relaxation. We also did not investigate the molecular mechanisms of endothelium-independent vasorelaxation which become impaired in preeclampsia and following MBG treatment. These questions as well as studies of the mechanisms of contractile effects of CTS in preeclampsia will be addressed in future work. An association of high plasma and placental levels of MBG, down-regulation of Fli-1 and

increased levels of procollagen-1 and collagen-1 in umbilical arteries with impairment of vascular relaxation found in the present study, suggests that MBG-induced fibrosis is responsible for this phenomenon. Previously we demonstrated that MBG-induced decrease in the levels of Fli-1 in a phospholipase C and PKC isoform-d-dependent manner leads to increases in collagen production in vitro and is implicated in the genesis of cardiac fibrosis in experimental uremic cardiomyopathy [15]. Fli-1, an Ets oncogene, competes with another transcription factor, ETS-1, to maintain a balance between stimulation and repression of Col1a2 gene promoter [32]. Reduced levels of Fli-1 are implicated in dermal fibrosis [33], but recently, we extended the pro-fibrotic role of Fli-1 to pathogenesis of uremic cardiomyopathy [15]. Thus, in rat and mouse cardiac and renal fibroblasts, concentrations of MBG which circulate in pathological conditions, suppress Fli-1 and stimulate synthesis of collagen [15]. Accordingly, in Fli-1 knockdown heterozygous mice expressing reduced cardiac collagen at baseline, effects of partial nephrectomy on cardiac levels of collagen-1 were much more pronounced than in their wild-type counterparts. Our present data agree with those previous observations and indicate that MBG-induced Fli-1-dependent collagen synthesis is implicated in one more scenario, induction of vascular fibrosis, which underlies impairment of vascular relaxation in preeclampsia.

In conclusion, our results indicate that in preeclampsia, elevated levels of MBG, via a Fli-1-dependent mechanism, stimulate synthesis of collagen in umbilical arteries, which leads to an impairment of vasorelaxation. Thus, MBG-induced Fli-1-dependent signaling pathway could contribute to vascular stiffness in preeclampsia. Because vascular stiffness plays an essential role in the development of vascular dysfunction in preeclampsia, MBG may represent a potential target for therapy of this syndrome.

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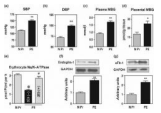

Figure 1.

Systolic (A) and diastolic (B) blood pressure, plasma (C) and placental (D) concentrations of MBG, activity of Na/K-ATPase in erythrocytes in subjects with uncomplicated pregnancy (N Pr) and with preeclampsia (PE) in the absence and in the presence of 3E9 anti-MBG monoclonal antibody (E), and placental levels of Endoglin-1 (F) and sFlt-1 (G): Upper panels – representative blots, lower panels - bars representing means ± SEM from 4 densiometry measurements. Levels of Endoglin-1, and sFlt-1 were normalized against levels of GAPDH. Means ± SEM. * - P<0.01; ** - P<0.001 vs. N Pr by two-tailed t-test. E: By one way ANOVA and Newman-Keuls test: @ - P<0.001 vs. N Pr; #-P<0.01 vs. 3E9(-).

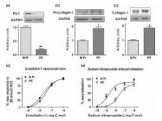

Figure 2.

Levels of Fli-1 (A), procollagen-1 (B), and collagen-1 (C) in the umbilical arteries from subjects with uncomplicated pregnancy (N Pr) and from patients with preeclampsia (PE). Upper panels – representative blots, lower panels - bars representing means ± SEM from 4 densiometry measurements. Levels of Fli-1, procollagen-1 and collagen-1 were normalized against levels of GAPDH. D – Vasoconstrictor effect of endothelin-1 in isolated rings of umbilical arteries. E – Vasorelaxant effect of sodium nitroprusside in isolated rings of umbilical arteries precontracted with 100 nmol/L endothelin-1. A-C: * - $P<0.01$; ** - $P<0.001$ by two-tailed t-test. D: $P>0.05$, N Pr vs. PE by repeated measures ANOVA followed by Newman-Keuls test. E: $P<0.01$, N Pr vs. PE by repeated measures ANOVA followed by Newman-Keuls test.

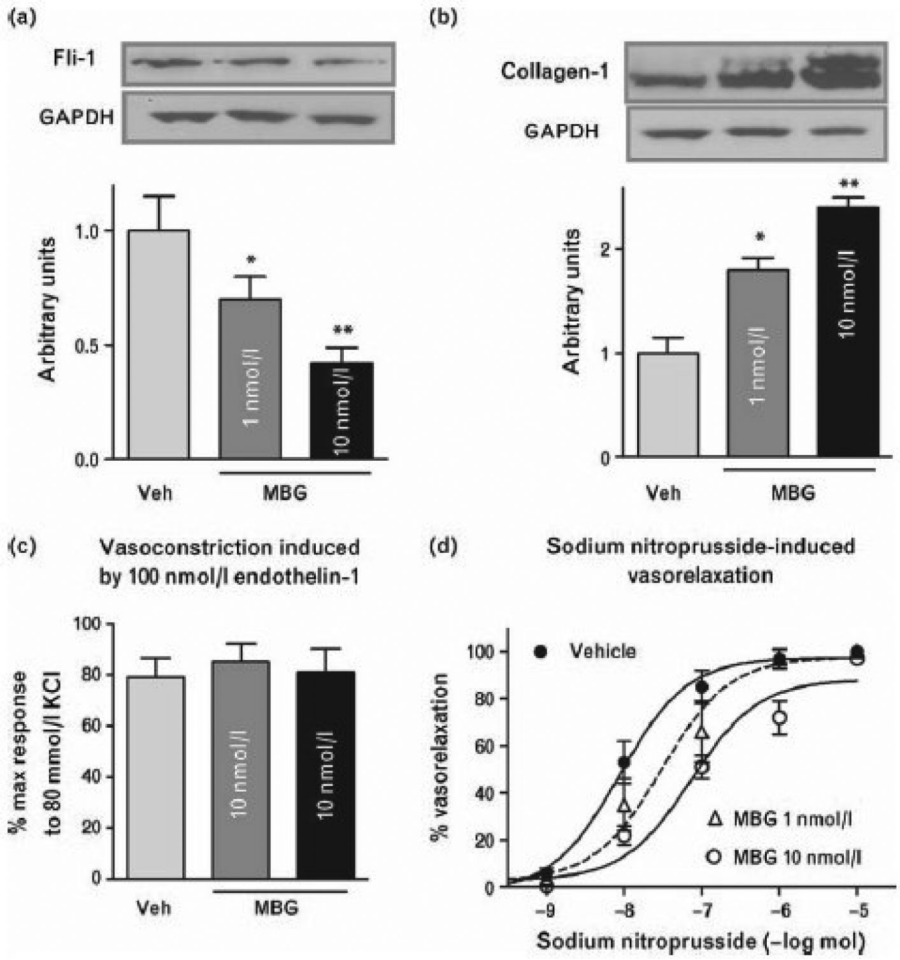

Figure 3.

Levels of Fli-1 (A) and collagen-1 (B) in the umbilical arteries from subjects with uncomplicated pregnancy treated for 24 hours by vehicle (VEH) and MBG (1 and 10 nmol/L). Upper panels – representative blots, lower panels - bars representing means ± SEM from 4 densiometry measurements. *-P<0.05, **-P<0.01 by one-way ANOVA followed by Newman-Keuls test. C – Vasoconstrictor effect of endothelin-1 (100 nmol/L) in isolated rings of umbilical arteries from subjects with uncomplicated pregnancy treated for 24 hours by vehicle and MBG (1 and 10 nmol/L). Levels of Fli-1 and collagen-1 were normalized against levels of GAPDH. D – Vasorelaxant effect of sodium nitroprusside in isolated rings of umbilical arteries from subjects with uncomplicated pregnancy treated for 24 hours by vehicle and MBG (1 and 10 nmol/L) and precontracted with 100 nmol/L endothelin-1. By repeated measures ANOVA followed by Newman-Keuls test: Vehicle vs. MBG 1 nmol/L – P=0.05; Vehicle vs. MBG 10 nmol/L – P<0.01.