Placental Na/K-ATPase inhibitor marinobufagenin induces arterial wall fibrosis in preeclampsia

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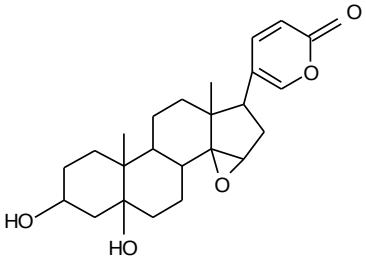
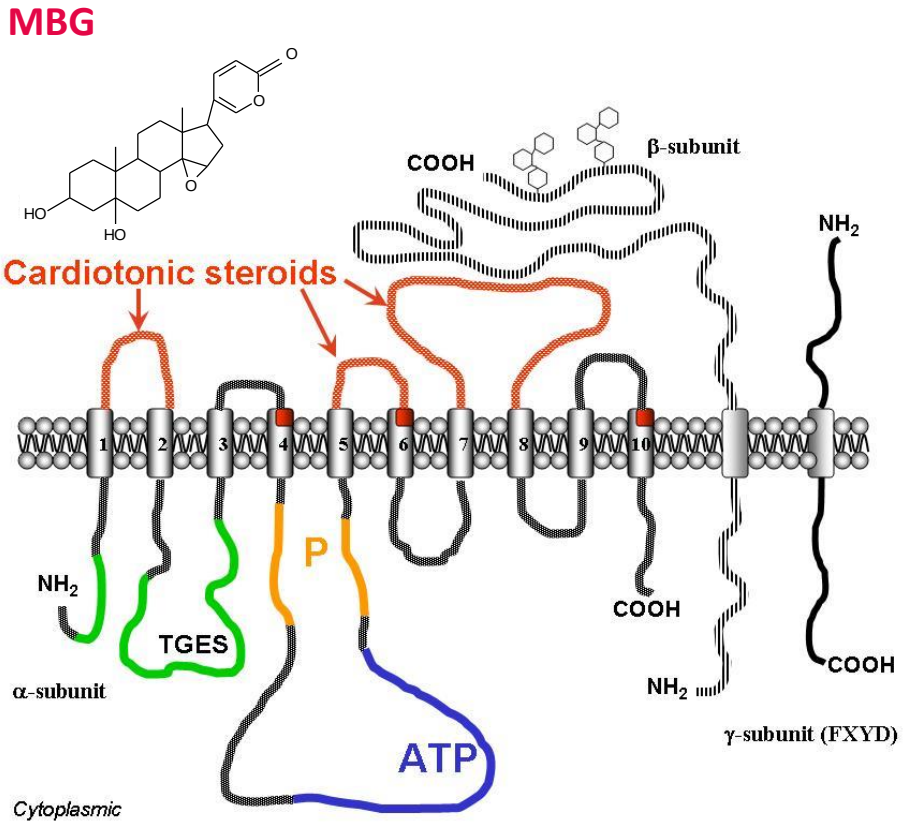
MBG is a selective inhibitor of α-1 isoform of Na/K-ATPase (NKA)



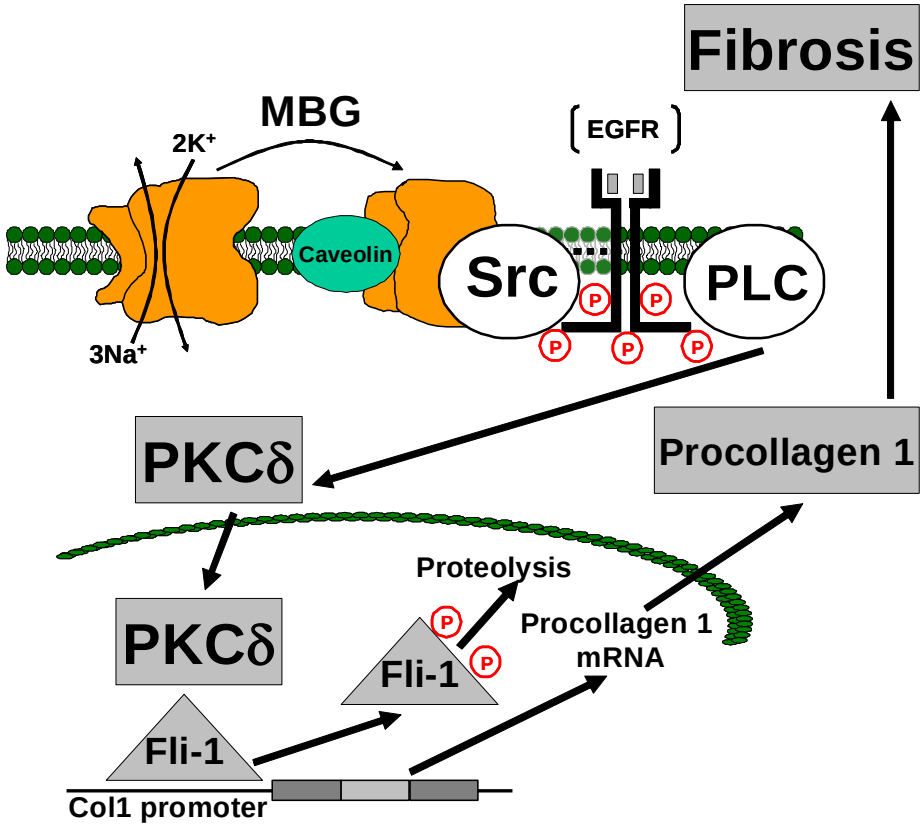
Bufo marinus

MARINOBUFAGENIN (MBG)
(Bufadienolide)



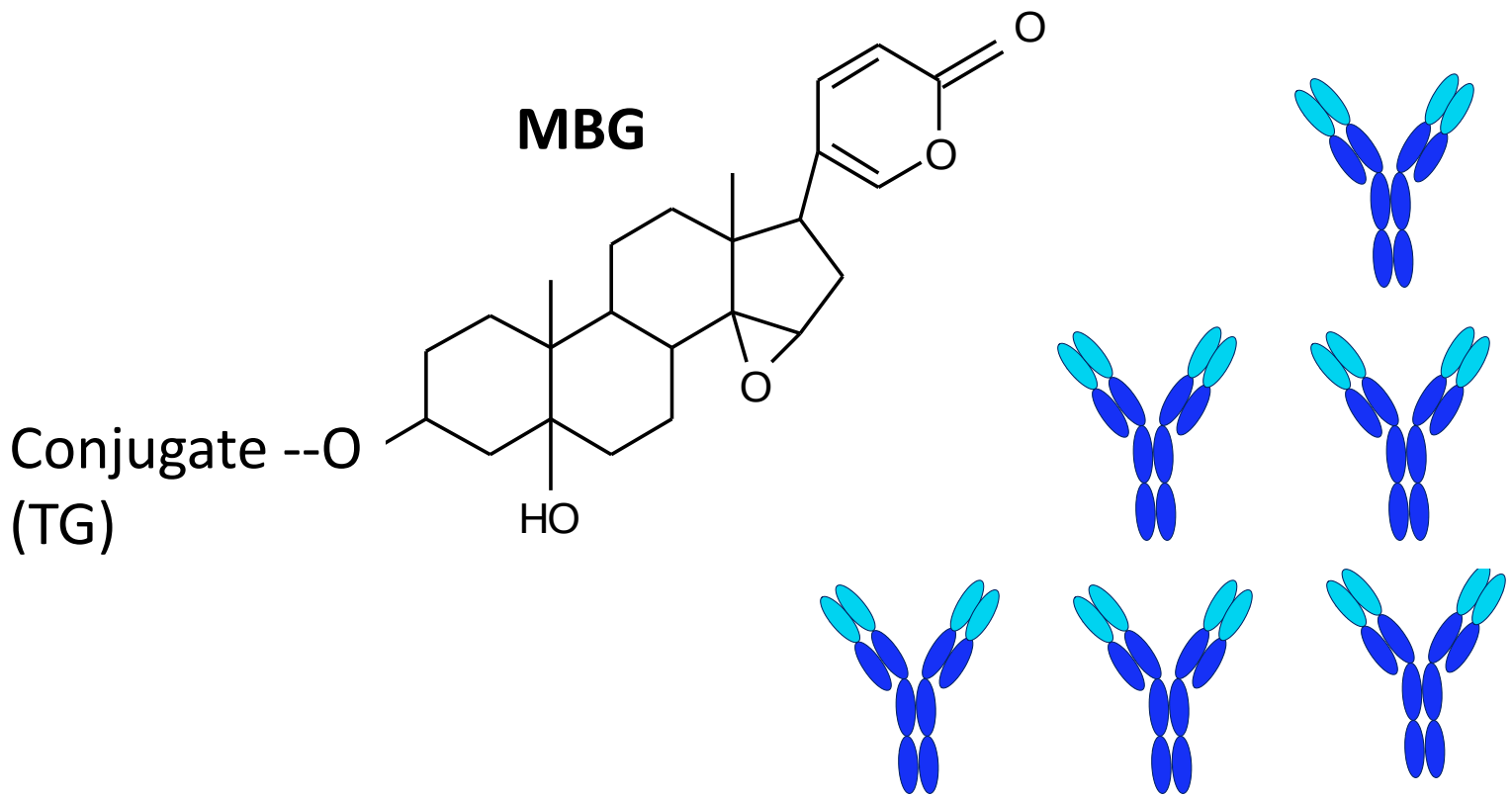
MBG is natriuretic hormone involved in salt balance, blood pressure regulation, and <u>fibrosis</u>.

Fibrosis is associated with high MBG and decreased Fli-1



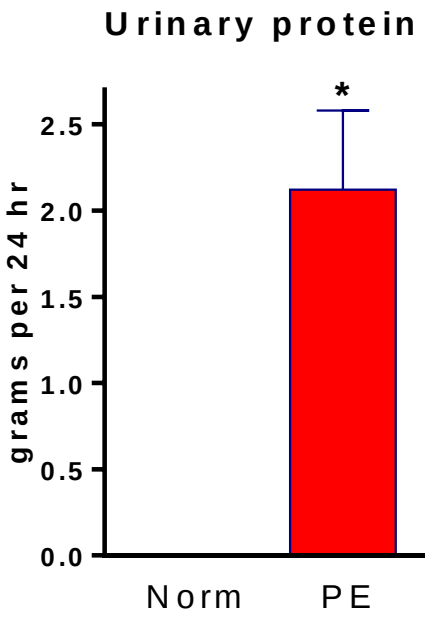
Fli-1, a member of ETS family, is a negative regulator of collagen synthesis. MBG via NKA inhibition phosphorylates Fli-1, which releases the collagen promoter, procollagen-1 is released, and collagen-1.

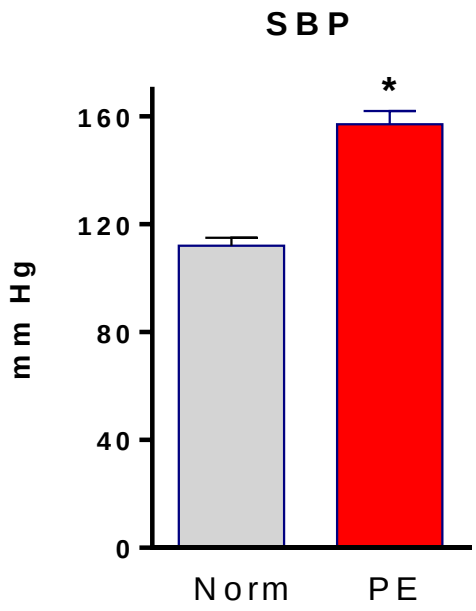
To test this we have designed monoclonal anti-MBG 3E9 neutralizing antibody:

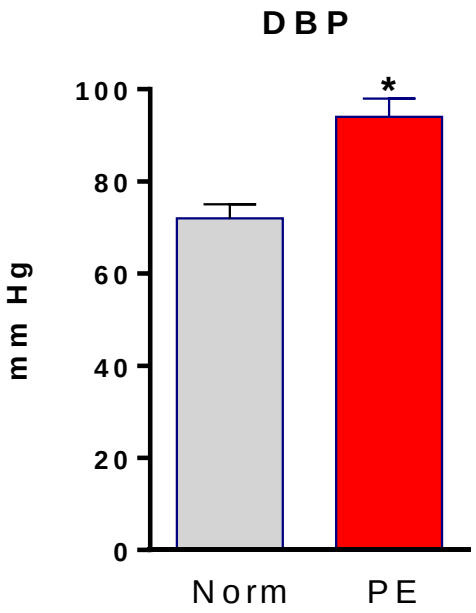


Study participants

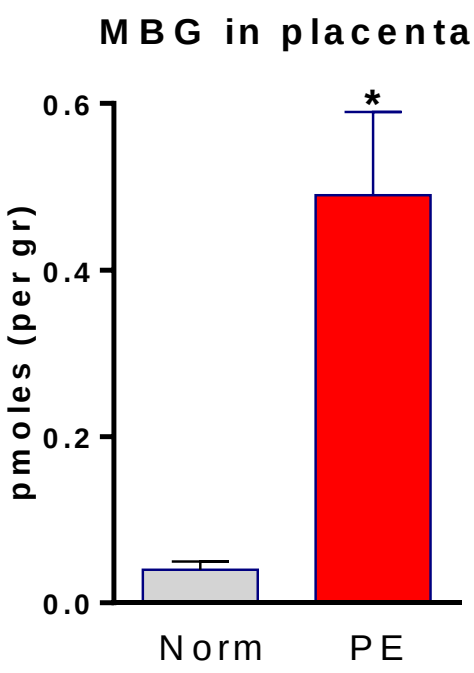
10 normotensive pregnant participants (gestational age: 39±0.4 weeks; BP 111±2 / 73±2 mmHg), and 11 patients with PE (gestational age: 39±0.5 weeks; BP: 156±5 / 94±2 mmHg) were enrolled. Placentae and umbilical artery explants were collected after delivery.

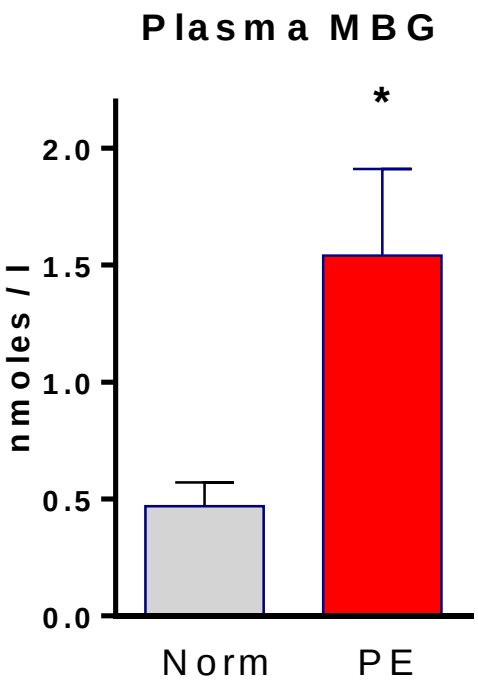






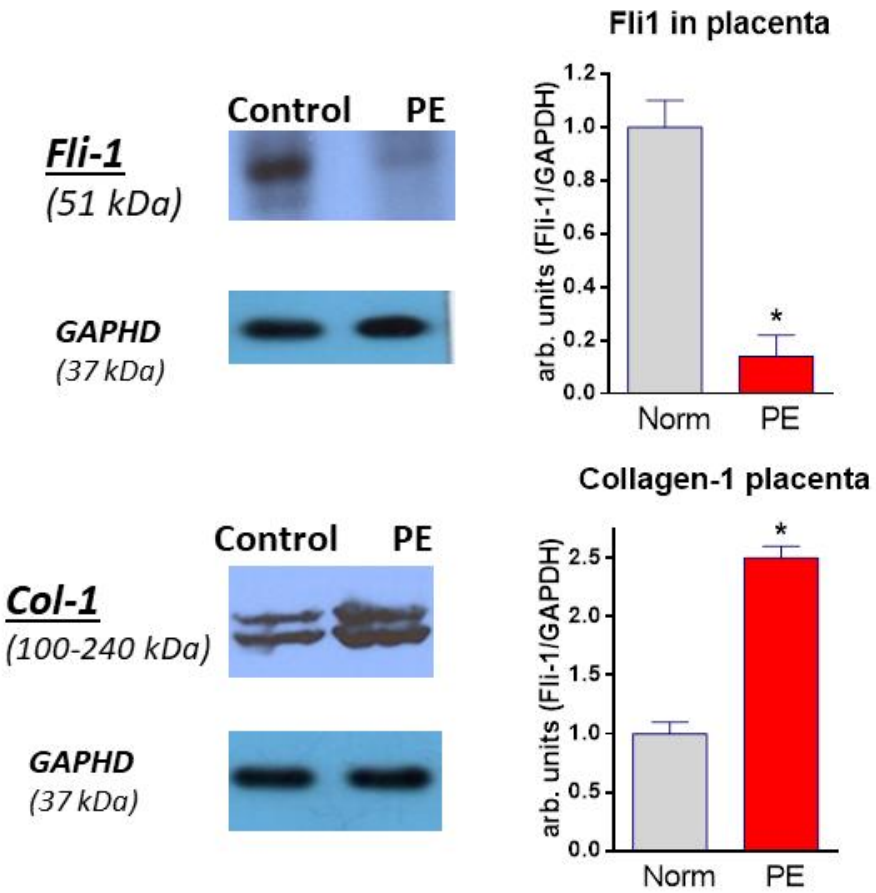
Levels of MBG in placenta of patients with PE exceeded those in plasma





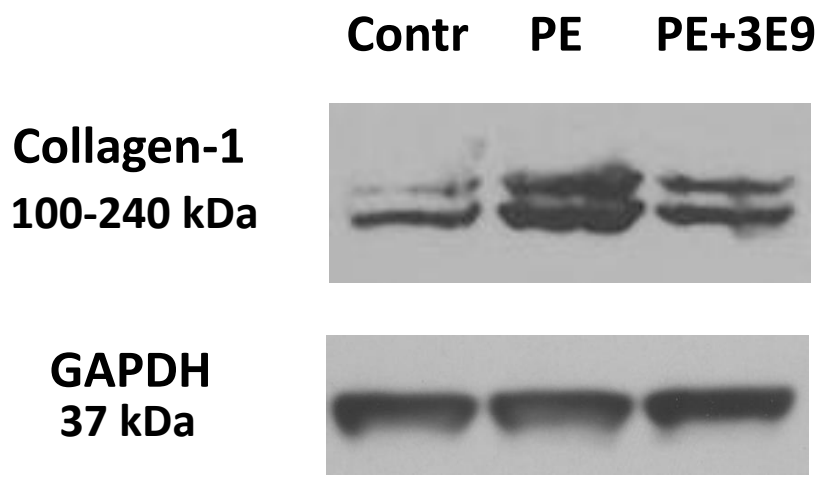
MBG in placenta exhibits 40-fold increase, versus in plasma 3.5-fold increase

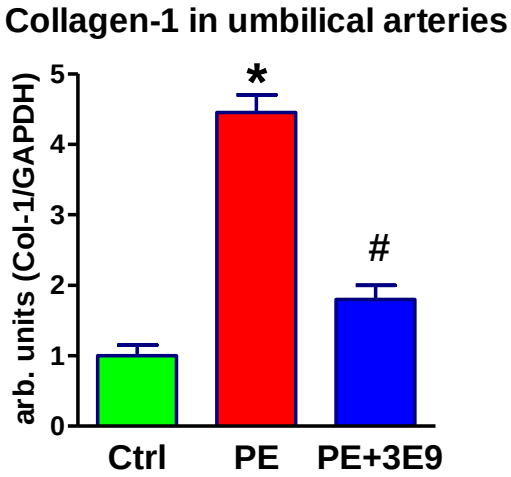
Explants of placentae from healthy human subjects and ones with PE were incubated with antibodies to Fli1 and Col-1. PE was associated with decreased levels of Fli1 and increased levels of collagen-1

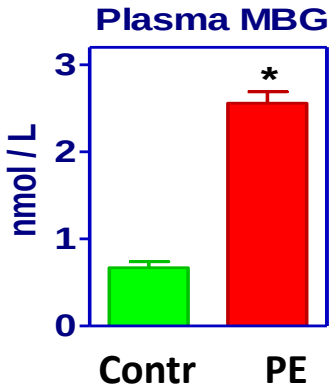


In explants of umbilical arteries from patients with preeclampsia, collagen-1 was higher than in control.

Addition of 3E9 anti-MBG antibody reduced collagen-1 (*ex vivo;* 24 hr).



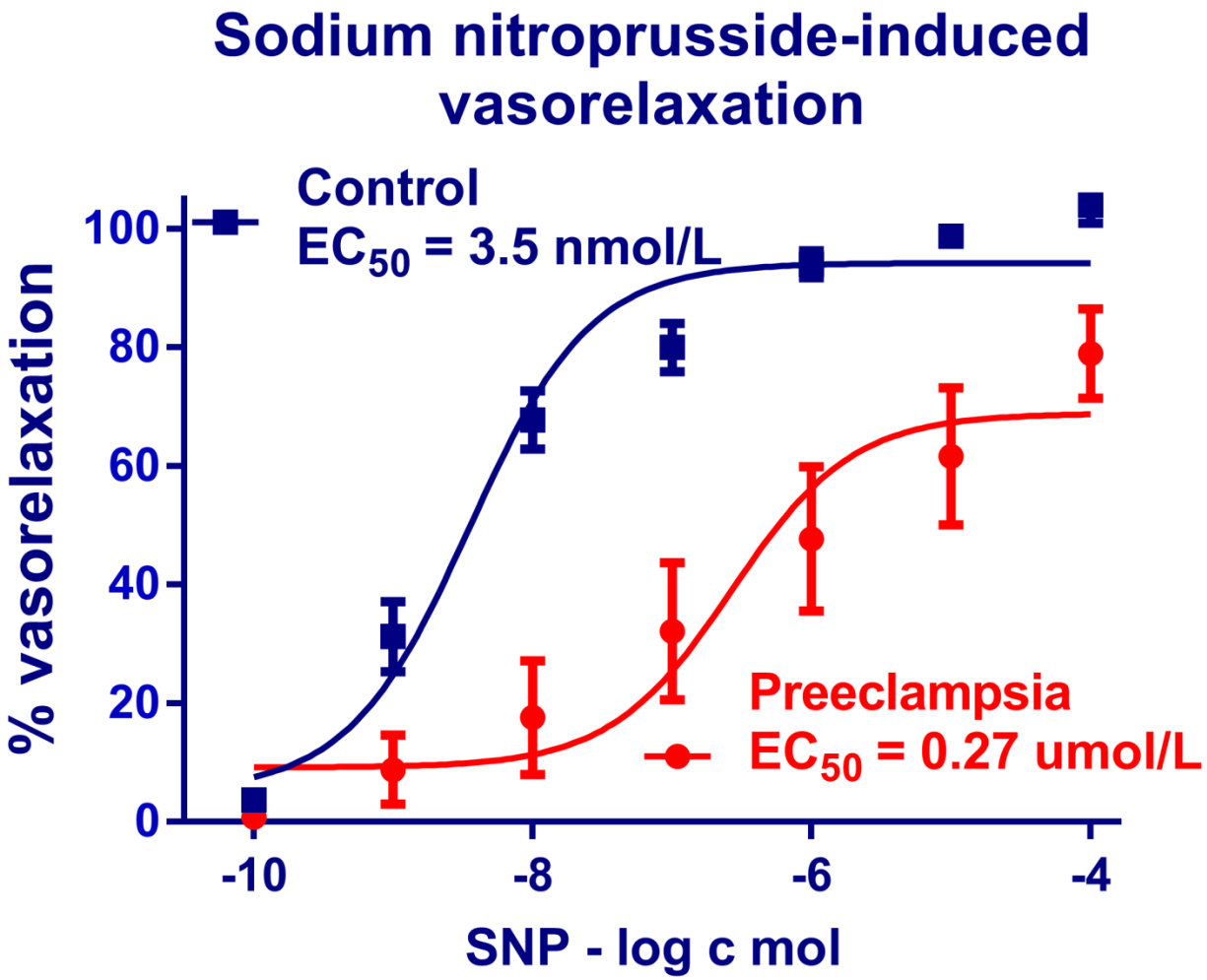




Thus, collagen is stimulated by MBG in preeclamptic patients.

Are the arteries fibrotic?

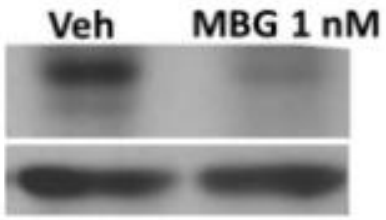
Explants of umbilical arteries from subjects with uncomplicated pregnancies contracted with Et-1 responded to SNP much better than those from patients with PE.



Can MBG induce fibrosis?
Explants of umbilical arteries from subjects with uncomplicated pregnancies were incubated with MBG for 24 h. MBG decreased levels of Fli-1 and increased levels of collagen-1:

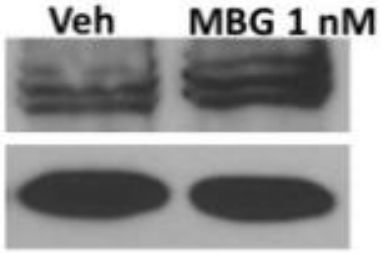
Fli-1 *(51 kDa)*

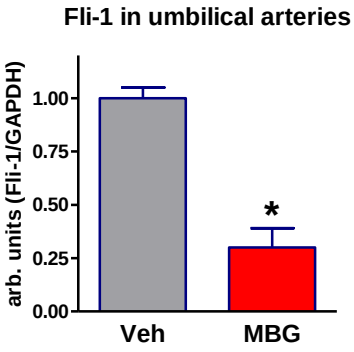
GAPHD *(37 kDa)*

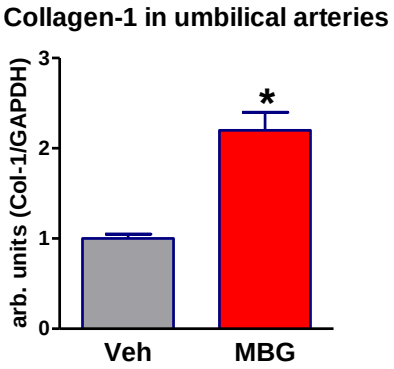


Collagen-1
(100-240 kDa)

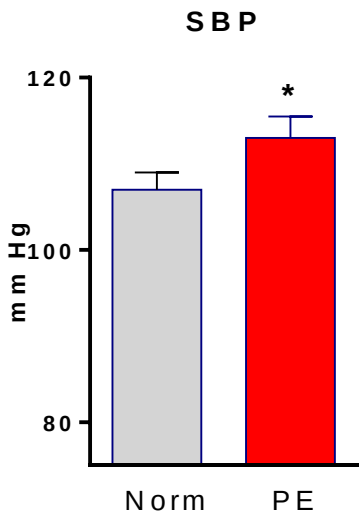
GAPDH *(37 kDa)*

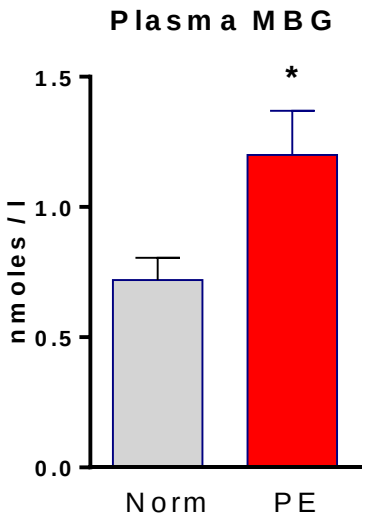


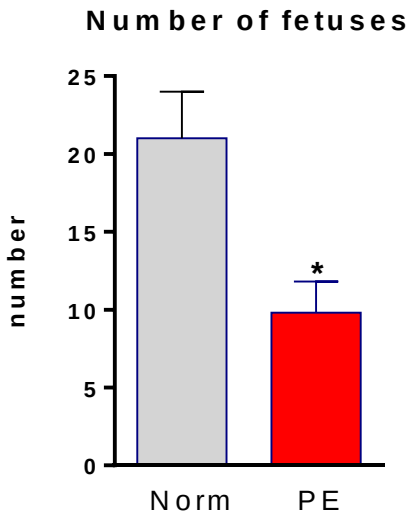


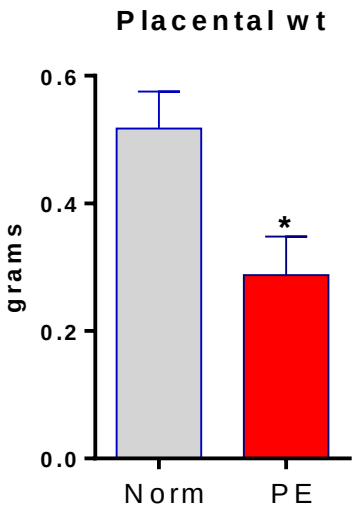


24 pregnant female Sprague-Dawley rats were studied. 8 rats were intact, 8 drunk 1.8% saline at days 11-21 of gestation, and 8 drunk 1.8% saline and were given a single injection of anti-MBG abs 3 hours before euthanasia.





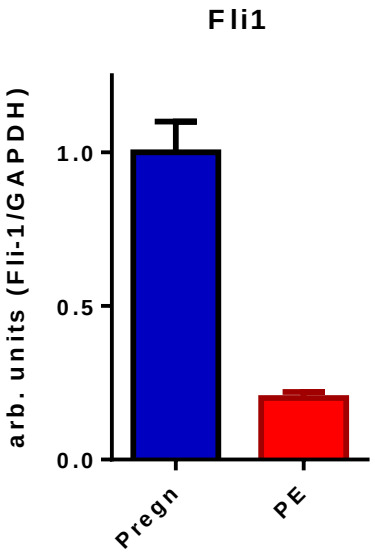


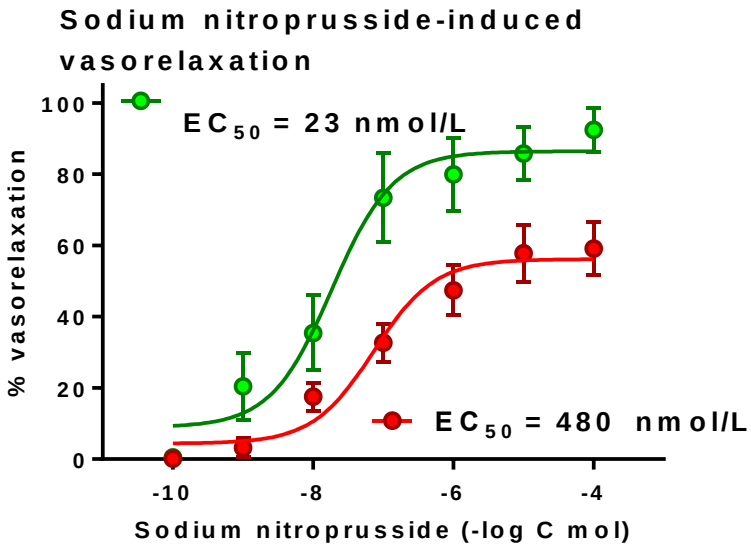


Umbilical arteries: In PE MBG decreased levels of Fli-1 and increased levels of collagen-1

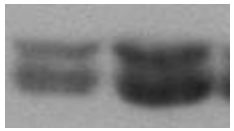
Fli-1 *(51 kDa)*

GAPHD *(37 kDa)*

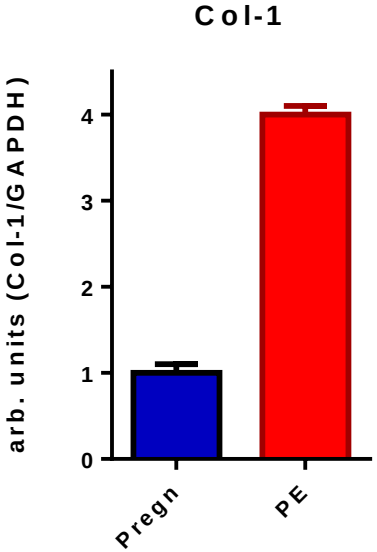




Collagen-1
(100-240 kDa)



GAPHD *(37 kDa)*

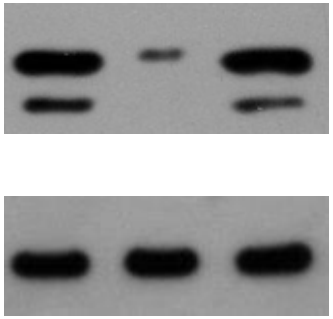


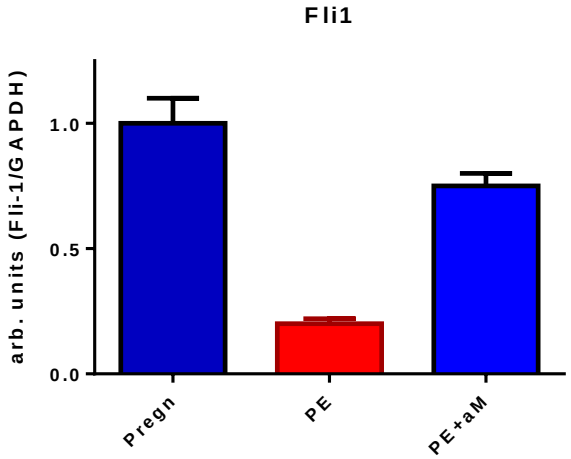
Explants of umbilical arteries from rats with uncomplicated pregnancies contracted with Et-1 responded to SNP much better than from rats with PE

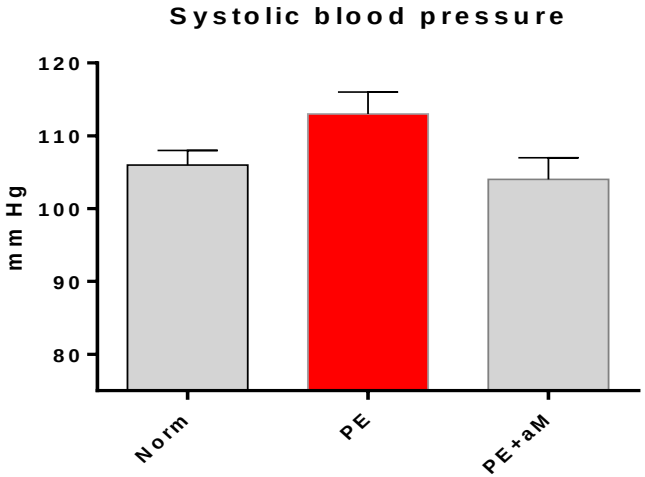
Development of preeclampsia in rat was associated with a drop of Fli1 in aorta. When “preeclamptic” rats were injected with anti-MBG antibody SBP decreased and that was associated dramatic increase of Fli1 in aortic sarcolemma.

Fli-1 *(51 kDa)*

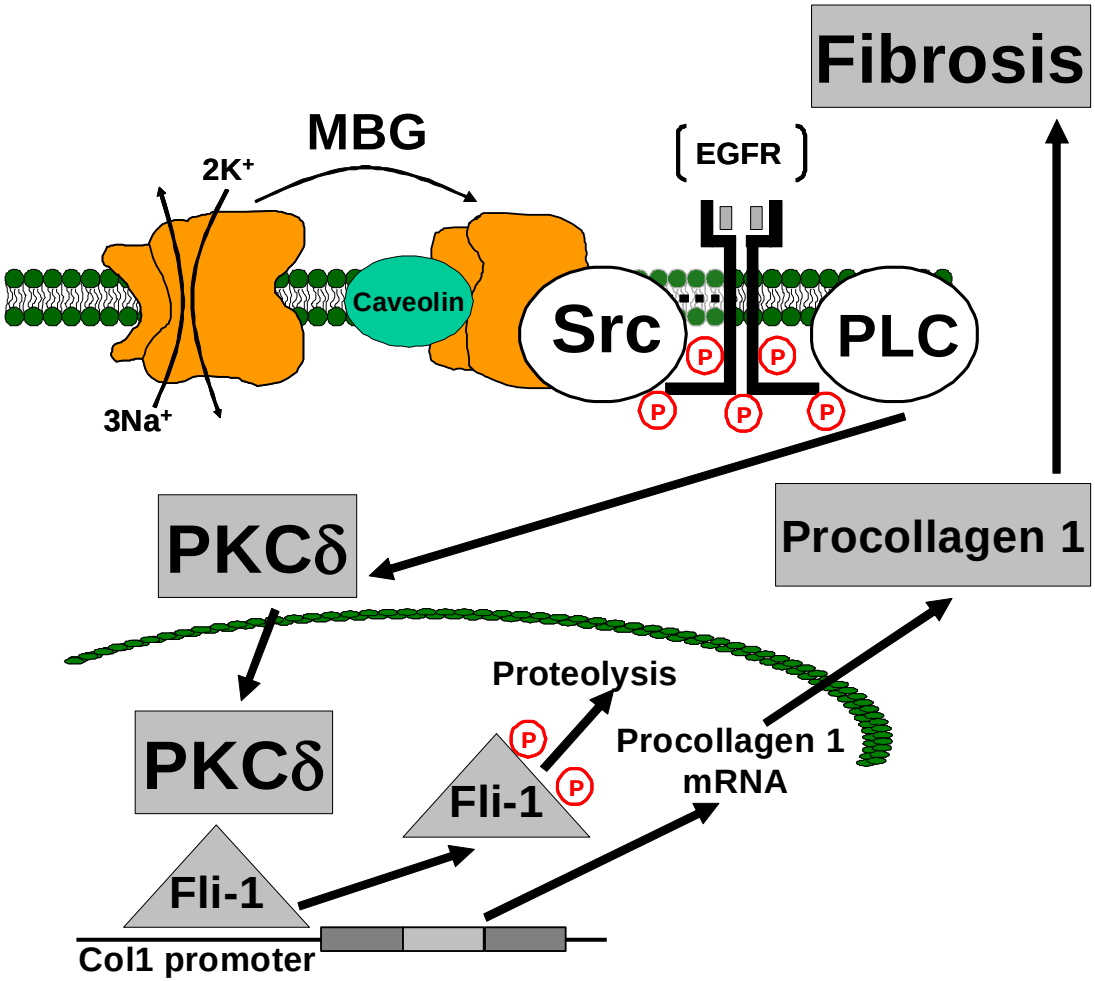
GAPHD *(37 kDa)*







Fli-1 implicated in pro-fibrotic signaling, was down-regulated in PE. Anti-MBG mAb treatment reversed this effect.



Conclusions

In preeclampsia MBG-induced pro-fibrotic effect is initiated in the placenta. MBG-induced vascular fibrosis causes impairment of vasorelaxation in umbilical arteries.

In preeclampsia elevated levels of MBG induce vascular fibrosis via a Fli-1-dependent mechanism.

These findings indicate a causative relationship between MBG and arterial stiffness.

Acknowledgements:

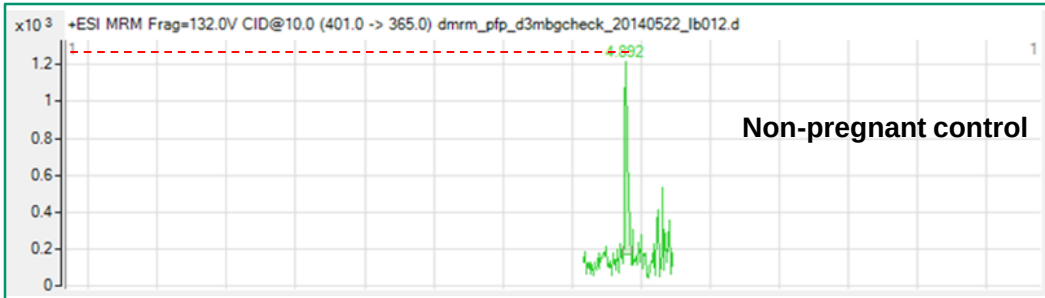
Almazov Federal Center, St. Petersburg, Russia
Valentina Ishkaraeva, MD
Irina Zazerskaya, MD, PhD

Pediatric School of Medicine, St. Petersburg, Russia
Natalia I Tapilskaya, MD, PhD

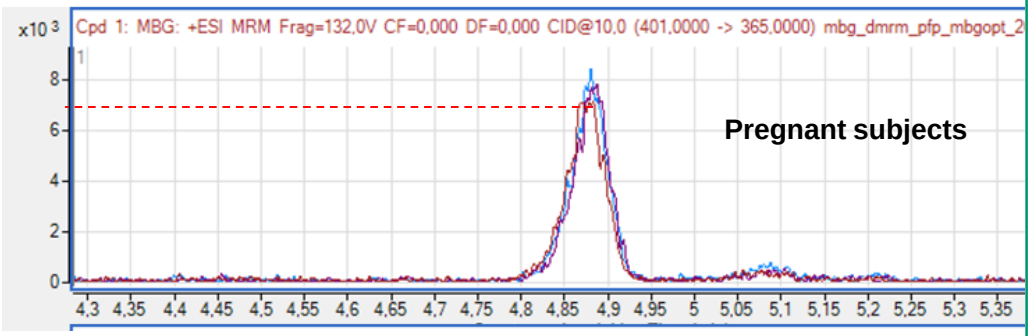
Sechenov Institute of Evolutionary Physiology, St. Petersburg, Russia
Olga Nadey, BSc

Supported by Russian Scientific Foundation grant № 18-15-00222

Mass-spectral analysis demonstrated increase of MBG level in plasma from normal pregnant subjects vs. control.



MS/MS Identification of MBG in non-pregnant plasma

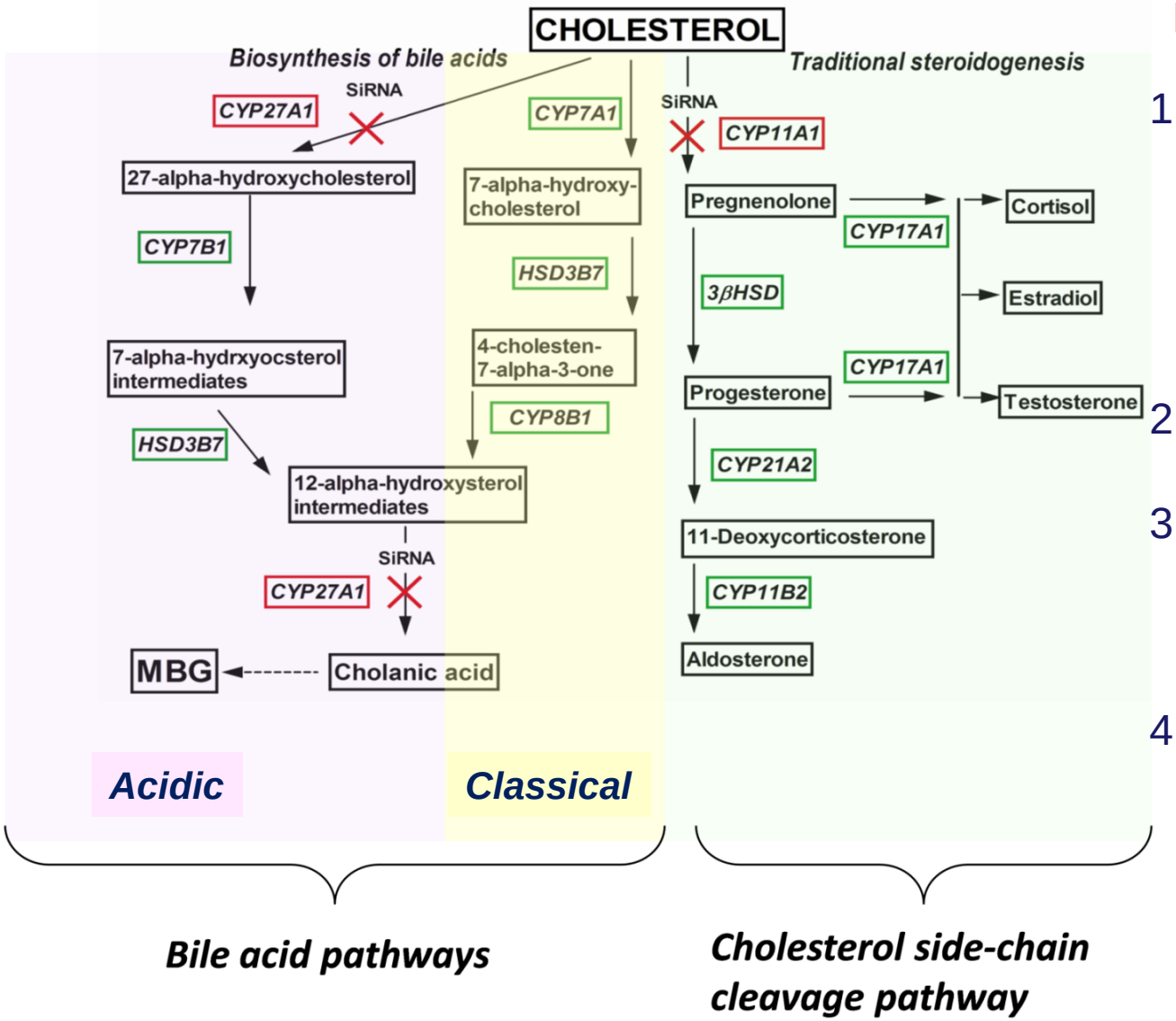


MS/MS Identification of MBG in plasma at 15 weeks pregnancy

Revealing of endogenous marinobufagenin by an ultra-specific and sensitive UHPLC-MS/MS assay in pregnant women.

Lenaerts C, Bond L, Tuytten R, Blankert B. Talanta. 2018 Sep 1;187:193-199

Two major pathways of steroidogenesis



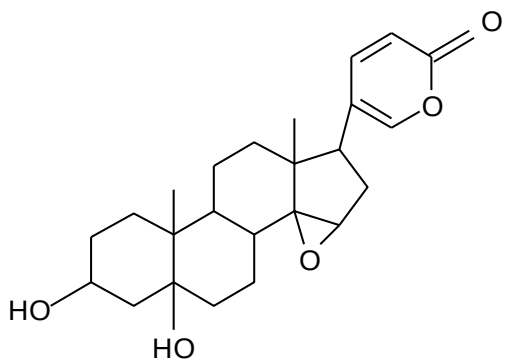
How is MBG synthetized?

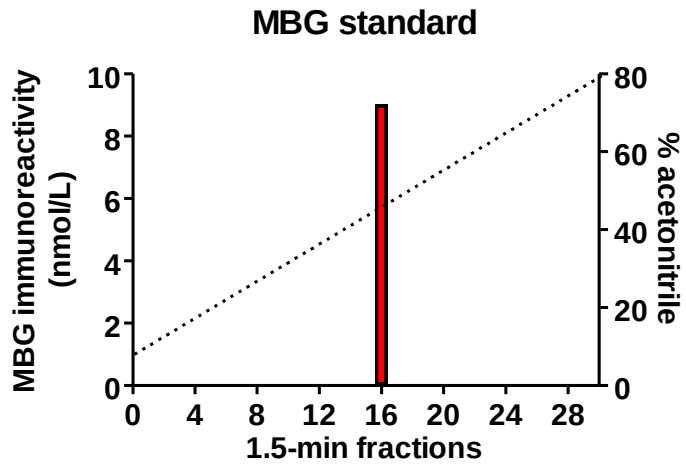
1. Side-chain cleavage of cholesterol, initiated by CYP11A1, is not involved, as previously demonstrated.
2. Bile acid pathways?
3. “Classical” pathway is not applicable, since it occurs in liver only.
4. “Acidic” pathway, initiated by **CYP27A1**, occurs in extrahepatic tissues, where MBG is produced. **CYP27A1** silencing.

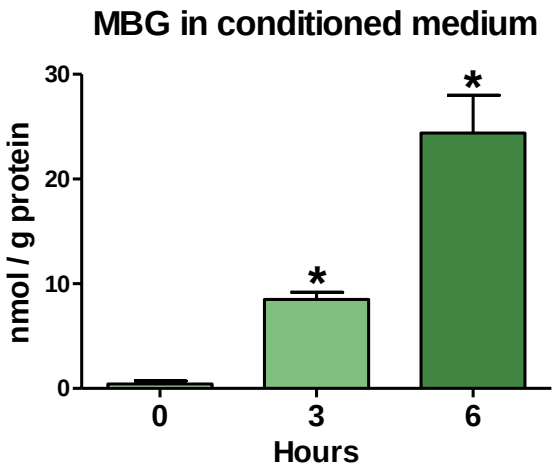
MBG standard was compared with HLPC-fractionated media from JEG-3 cells. Placental JEG-3 cells produce MBG, which is indistinguishable from MBG standard.

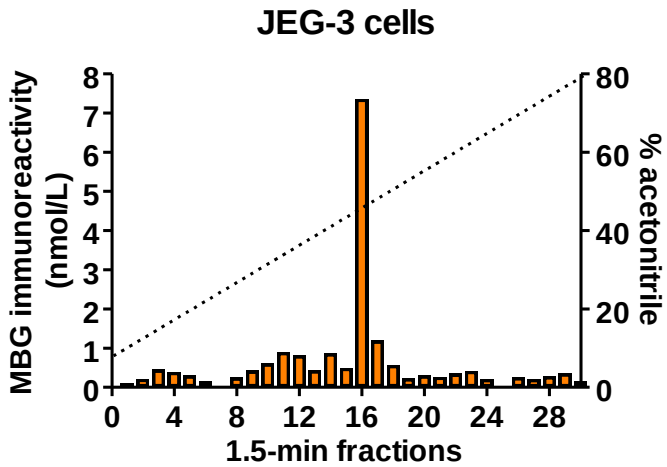
Marinobufagenin

3β,5β-dihydroxy-14,15-epoxy bufadienolide

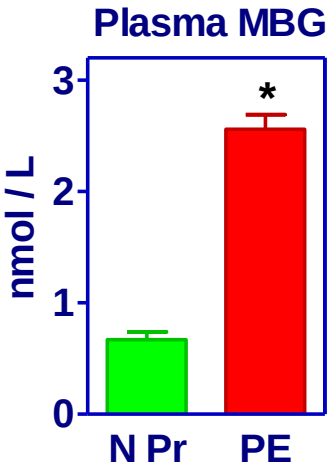


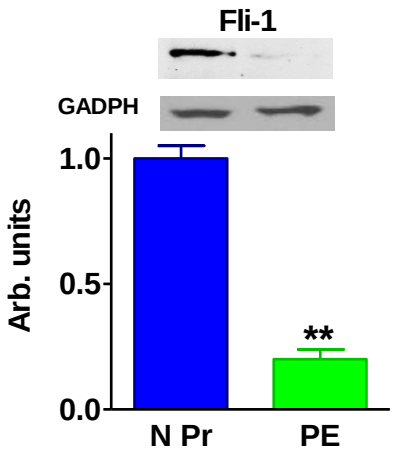


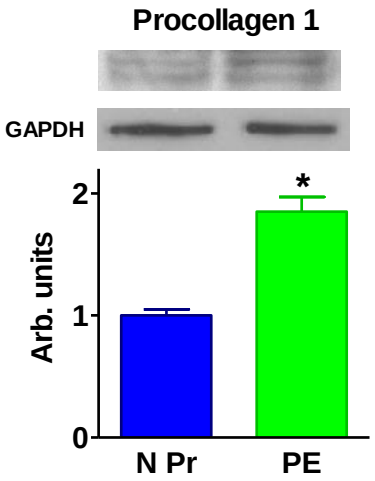


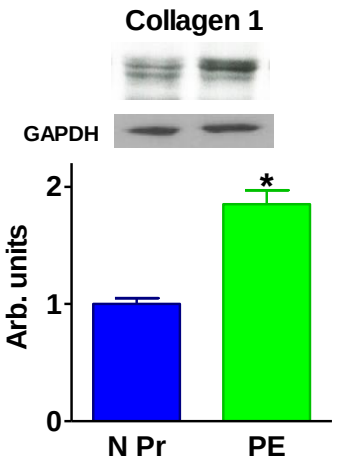


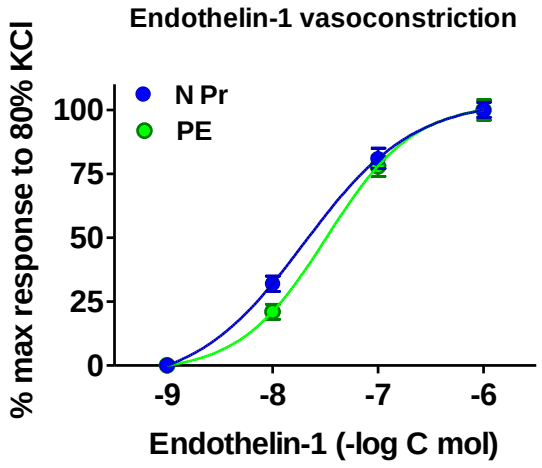
Umbilical arteries in preeclampsia: Unaltered contractile responses, but markedly impaired relaxation

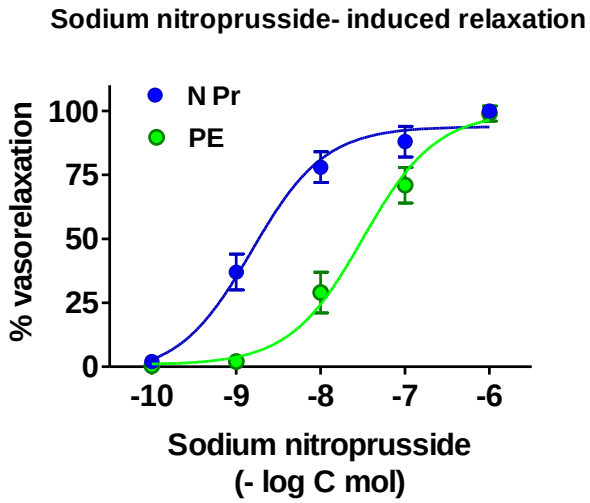






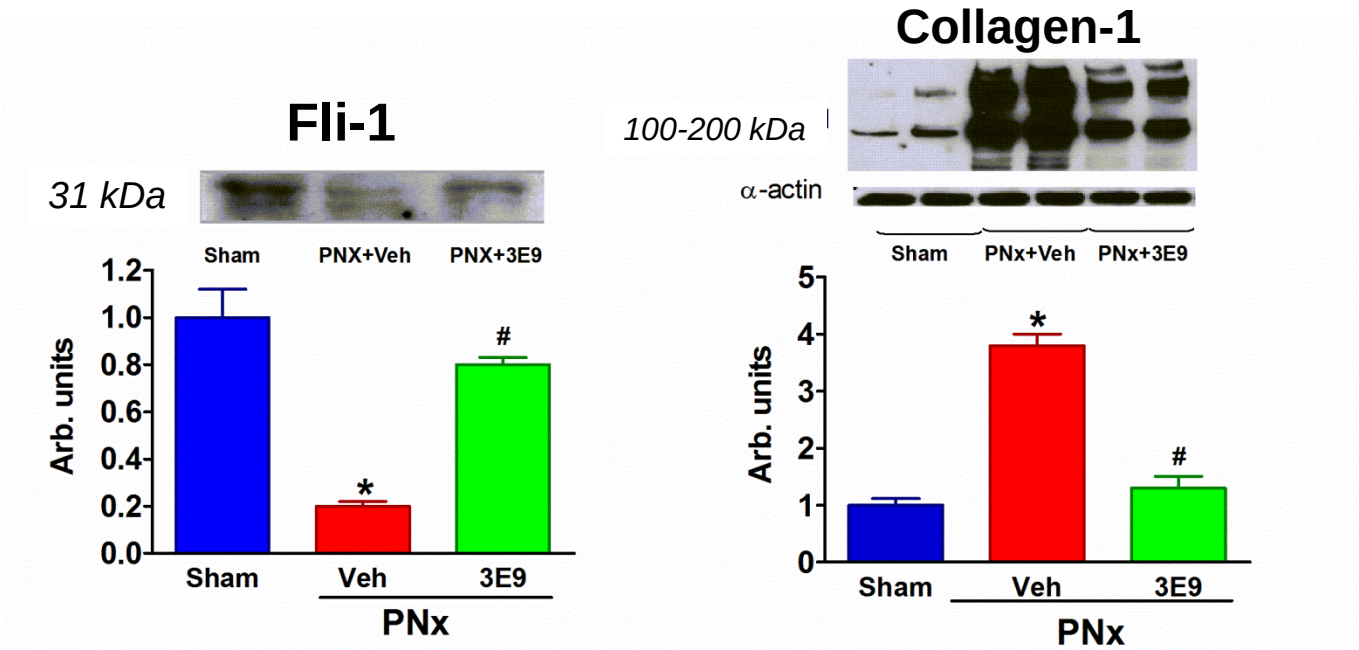




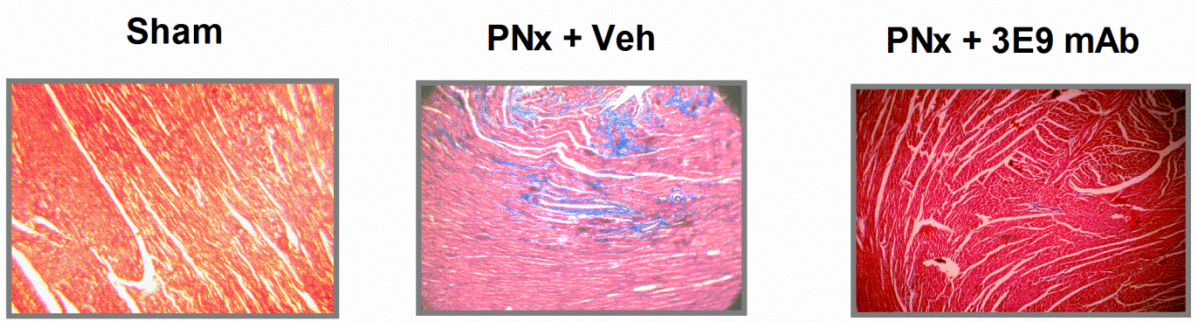


Nikitina et al, J Hypertens, 2011

In left ventricles from CRF (PNx) rats, level of the Fli-1 decreased and the level of collagen-1 increased. Administration of 3E9 anti-MBG mAb restored Fli-1 and reduced levels of collagen-1 and fibrosis:



PNx – partial nephrectomy



Haller et al, Am J Hypertens 2012

Collagen is stained blue

MBG increases:

- In salt sensitive hypertension
- In heart failure
- In acute myocardial infarction
- In chronic renal diseases
- In preeclampsia

Bagrov et al, Pharmacol Rev, 2009