Final report

Title of the project: The role of the endocannabinoid system in the development of preeclampsia (PD 109094)
Duration: 2013 Sep 1- 2015 Aug 31

Preeclampsia (PE) is one of the most common complications of pregnancy and a major cause of maternal and perinatal morbidity and mortality. Despite intensive research, the etiology of the disease is still unknown, and the only definitive treatment is delivery. The endocannabinoid system (ECS) plays a role in regulation of reproductive functions (implantation, decidualization, placentation) and blood pressure and development of cardiovascular risk factors (ie. obesity, diabetes, lipid metabolism disorder and atherosclerosis). These factors are strongly associated with preeclampsia. The aim of our project was to determine the link between PE and ECS. We measured serum levels of the ECS molecule anandamide (AEA), the anti-angiogenic soluble fms-like tyrosine kinase-1 (sFlt-1) and angiogenic placental growth factor (PlGF), along with the expression of the cannabinoid receptors (CB1, CB2) and the enzyme responsible for AEA degradation in human placenta.

I. Placental expression of the endocannabinoid system in preeclampsia

In the first year of our project, we analyzed cannabinoid receptor 1 (CB1), cannabinoid receptor 2 (CB2) and fatty acid amid hydrolase (FAAH) expressions and localization in normal and preeclamptic placenta, in order to determine whether aberrant endocannabinoid activity is related to preeclampsia. Eighteen preeclamptic patients and 18 normotensive, healthy pregnant women with uncomplicated pregnancies were involved in this case-control study. We determined CB1, CB2 and FAAH expressions by Western blotting and immunohistochemistry in placental samples collected directly after Cesarean section. For statistical analyses, non-parametric methods were applied. Placental expression of CB1 protein measured by Western blotting was significantly higher in preeclamptic patients than in normotensive, healthy pregnant women (149.3 (105.0-279.7) % versus 98.1 (67.3-131.0) %, $p=0.008$). Nevertheless, no significant differences were observed in placental CB2 (105.5 (80.9-133.2) % versus 65.8 (45.5-128.4) %, $p>0.05$) and FAAH (112.2 (94.7-143.8) % versus 91.2 (63.3-128.4) %, $p>0.05$) protein expressions between the two study groups. According to

immunohistochemistry, CB1 immunoreactivity was markedly stronger in syncytiotrophoblasts, the mesenchymal core, villous capillary endothelial and smooth muscle cells, as well as in the decidua and amnion in preeclamptic samples compared to normal pregnancies. However, we did not find a difference between preeclamptic and normal placenta in terms of CB2 and FAAH immunoreactivity. In conclusion, we observed markedly higher expression of CB1 protein in preeclamptic placental tissue. Increased CB1 expression might cause abnormal decidualization and impair trophoblast invasion, thus being involved in the pathogenesis of preeclampsia. As CB1 activation can induce endothelial dysfunction and enhance vascular inflammation, the strong CB1 immunoreaction in vascular endothelial and smooth muscle cells suggests that CB1 may contribute to the development of atherosis in the placental villi shown earlier in preeclampsia. While the detailed pathogenesis of preeclampsia is still unclear, our results suggest that the endocannabinoid system might play a role in the development of the disease.

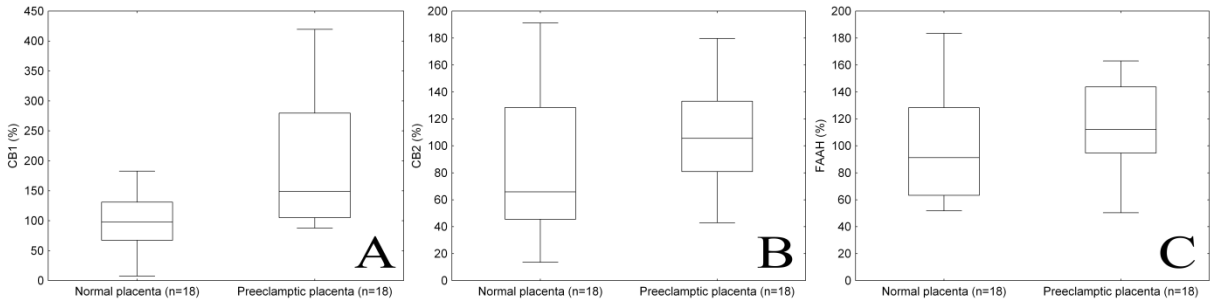

Figure 1.

Densitometry analysis of CB1 ($p=0.008$, A), CB2 ($p>0.05$, B) and FAAH ($p>0.05$, C) expressions in normal and preeclamptic placental tissue.

Middle line: median; Box: interquartile range (25-75 percentile); Whisker: range (excluding outliers).

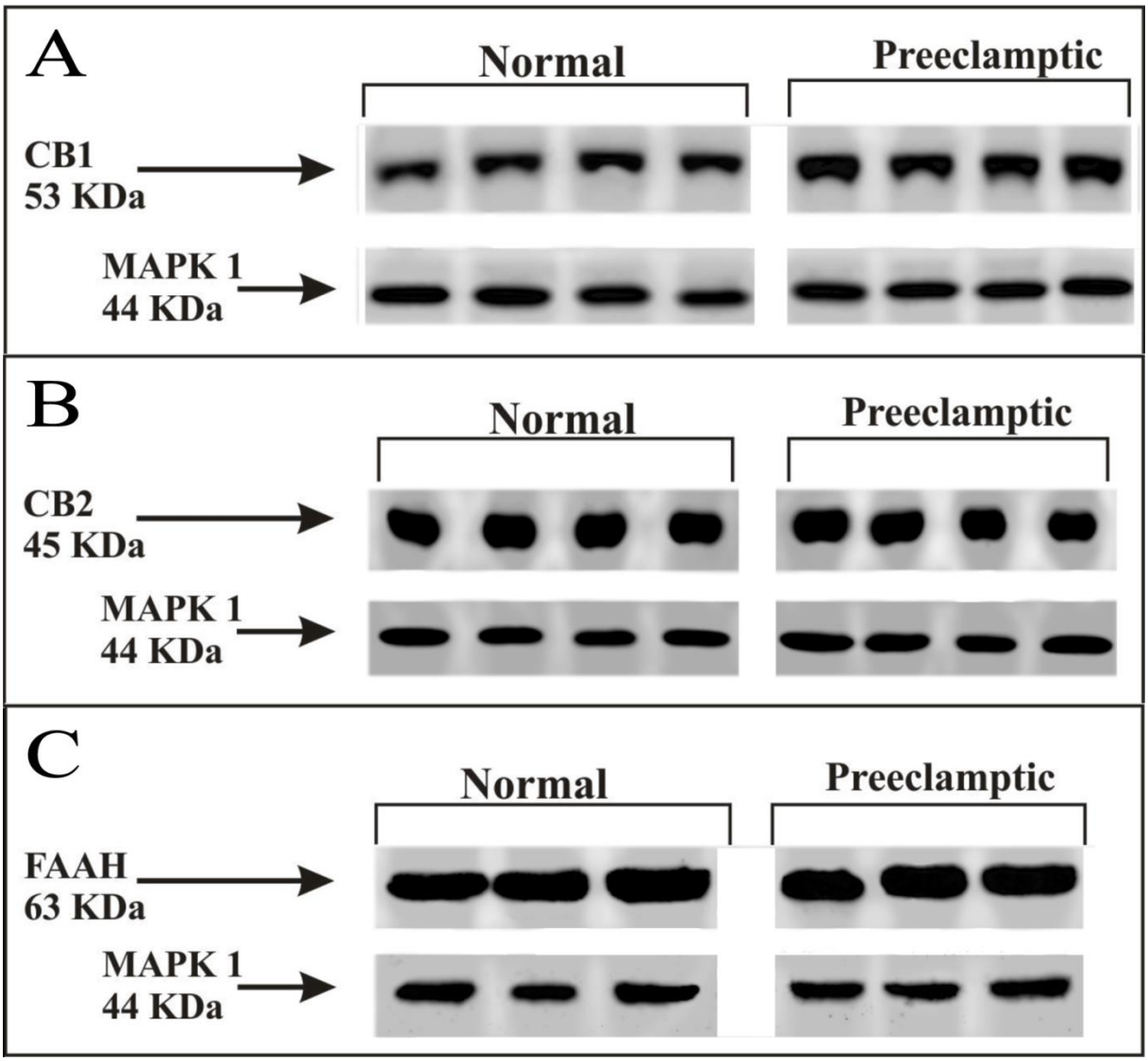

Figure 2.

Representative Western blotting demonstrating CB1 (A), CB2 (B) and FAAH (C) expressions in normal and preeclamptic placental tissue.

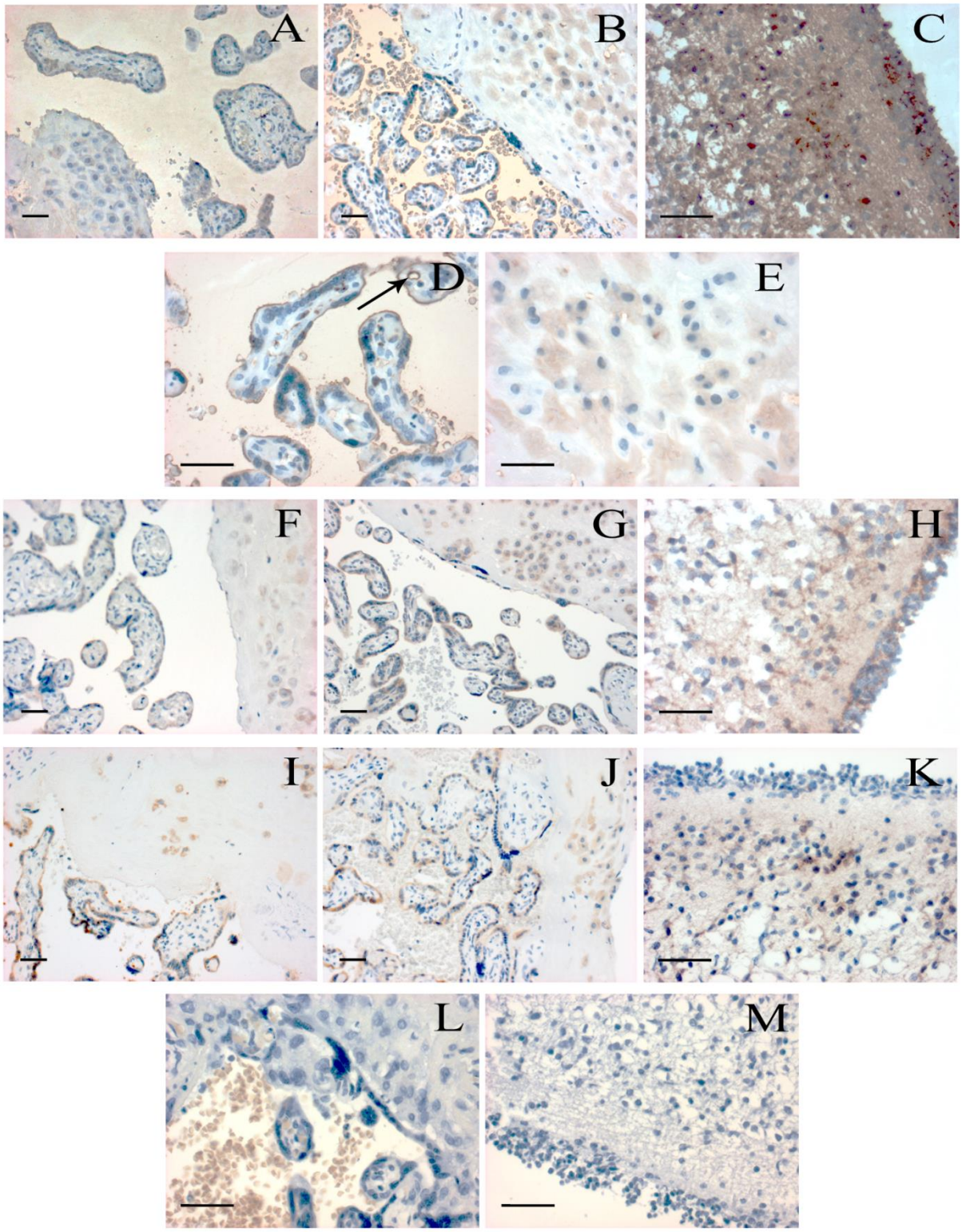

Figure 3.

Localization of CB1, CB2 and FAAH in human placental villi, decidua and cerebellum.

CB1 specific staining is shown in normal (A) and preeclamptic (B) placental tissues, images were captured at 200× magnification. Further images demonstrate strong CB1 positivity in preeclamptic placental villi (D) and decidua (E) at 400× magnification. Arrow indicates

strong CB1 immunoreactivity in villous capillary endothelial cells. Positive control reaction for CB1 is revealed in human cerebellum (C).
CB2 specific staining is shown in normal (F) and preeclamptic (G) placental tissues, and in human cerebellum (H) as a positive control.
FAAH specific staining is presented in normal (I) and preeclamptic (J) placental tissues, and in human cerebellum (K) as a positive control.
Negative controls are shown in human placental villi and decidua (L), and in human cerebellum (M).
Placental tissue samples from normal pregnancy and preeclampsia were captured at 200× magnification, while positive and negative controls were taken at 400× magnification; bar = 100 μm.

II. Circulating anandamide levels in preeclampsia

In the second year of our project, we measured circulating levels of anandamide along with those of sFlt-1 and PlGF in normal pregnancy and preeclampsia. Forty-three preeclamptic patients and 71 healthy pregnant women were involved in this case-control study. Serum anandamide concentrations were determined by high performance liquid chromatography-mass spectrometry (HPLC-MS) technique. Serum total sFlt-1 and biologically active PlGF levels were measured by electrochemiluminescence immunoassay. For statistical analyses, non-parametric methods were applied. Serum levels of anandamide were significantly lower in preeclamptic patients than in healthy pregnant women (0.75 (0.44-1.03) ng/ml versus 1.30 (0.76-2.0) ng/ml, $p<0.001$). Preeclamptic patients had significantly higher sFlt-1 levels (12121 (7963-18316) pg/ml versus 2299 (1393-3179) pg/ml, $p<0.001$) and significantly lower PlGF concentrations (71.2 (39.2-86.4) pg/ml versus 256.8 (181.1-421.0) pg/ml, $p<0.001$) as compared to healthy pregnant women. Serum anandamide concentrations did not correlate with serum levels of sFlt-1 and PlGF in our healthy pregnant and preeclamptic groups. Decreased serum concentration of anandamide in preeclampsia might be a consequence of its sequestration in the placenta by binding to syncytiotrophoblasts overexpressing CB1. As endocannabinoids have been reported to exert anti-inflammatory effects, decreased circulating anandamide levels might contribute to the development of the excessive systemic inflammatory response and the increase in the ratios of peripheral blood Th1/Th2 and Th17/regulatory T cells characteristic of the maternal syndrome of the disease. In addition, chronic activation of the endocannabinoid system results in decrease of blood pressure. Thus,

it is also possible that decreased serum anandamide concentration might play a role in blood pressure elevation in preeclampsia. In our study, increased sFlt-1 and decreased PlGF levels were not related to serum anandamide concentrations in women with preeclampsia, suggesting that alterations in circulating levels of angiogenic factors and anandamide are independent processes in preeclampsia.

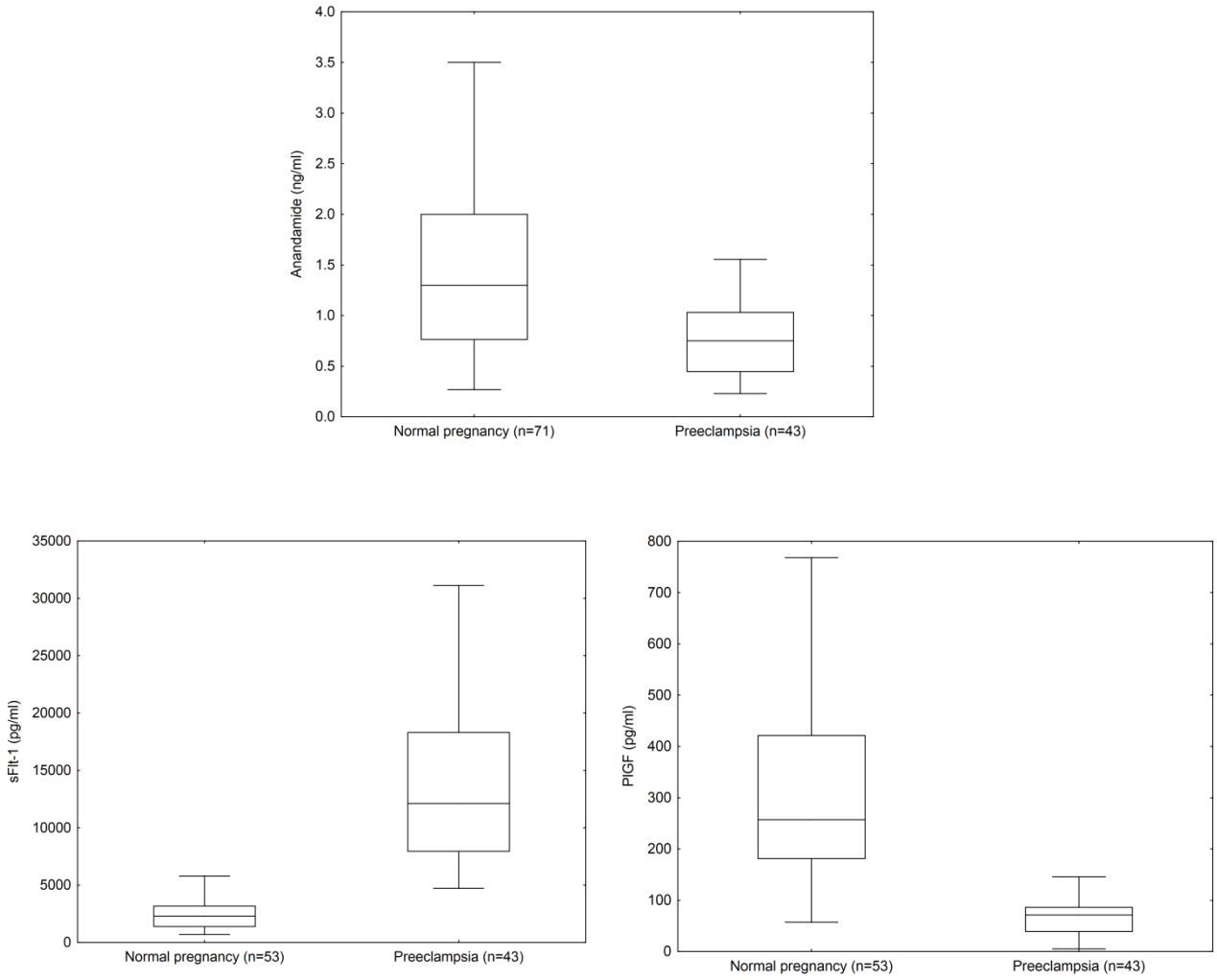

Figure 4.

Serum anandamide, soluble fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PlGF) levels of healthy pregnant women and preeclamptic patients ($p<0.001$)

Middle line: median; Box: interquartile range (25-75 percentile); Whisker: range (excluding outliers)