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Elevated levels of neuropeptide Y in preeclampsia: A pilot study implicating a role for stress in pathogenesis of the disease

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ABSTRACT

Objective: To determine if preeclampsia (PE) is associated with dysregulation of the neuropeptide Y (NPY) system.
Methods: The study enrolled 114 subjects either with normal pregnancy (NP) or with PE. Systolic blood pressure (SBP) was collected from patients using a standard sphygmomanometer. The PE patients were divided into two groups based on the gestational age (GA) at delivery — placental PE (PLPE, GA < 34 weeks) or maternal PE (MTPE, GA ≥ 34 weeks). NPY was measured in platelet rich plasma (PRP), platelet poor plasma (PPP) and in the serum of NP and PE patients utilizing radioimmunoassay. Serum levels of soluble fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PlGF) were measured in NP and PE subjects by ELISA.
Results: SBP was higher in PE compared to NP. Circulating NPY in serum and PRP, as well as NPY content per 100,000 platelets, but not its concentrations in PPP, were elevated in PE, as compared to NP. The highest NPY concentrations were observed in sera and PRP of patients with MTPE. PE patients had also elevated levels of sFlt-1, as compared to NP, although no difference between PLPE and MTPL groups were observed. There was no increase in P1GF in PE patients.
Conclusion: Systemic NPY is elevated in PE patients, as compared to NP. This increase is observed in blood fractions containing platelets, suggesting accumulation of the peptide in these cells. NPY concentrations are particularly high in patients with MTPE, underlying differences in etiology between PLPE and MTPE. Our study implicates NPY as a potential target in antihypertensive therapies for PE patients.

1. Introduction

The term preeclampsia (PE) refers to the new onset of hypertension (blood pressure higher than 140/90 mm Hg) and proteinuria after 20 weeks of gestation in previously normotensive, nonproteinuric women (Conrad and Lindheimer, 1999). It is a unique form of human hypertension, one of the most common medical complications of pregnancy, and associated with increased maternal and neonatal morbidity and mortality (NHLBI, 2000). Although the pathophysiologic mechanisms underlying PE still remain unclear, this condition is characterized by a marked increase in peripheral vascular resistance, leading to an increase in blood pressure that returns to normal after delivery (Cunningham and Lindheimer, 1992; Roberts and Redman, 1993; Schobel et al., 1996). Moreover, numerous studies have demonstrated decreased levels of angiogenic factors – vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) – in the blood of women with PE (Reuvekamp et al., 1999; Torry et al., 1998). This deficit in angiogenic factors is associated with high systemic concentrations of soluble fms-like tyrosine kinase-1 (sFlt-1), a releasable splice variant of the VEGF receptor type 1 (Maynard et al., 2003). Circulating sFlt-1 binds and deactivates VEGF and PlGF, depriving the endothelium of these essential growth factors and acting as an antiangiogenic molecule (Karumanchi and Bdolah, 2004). PE can be divided into 2 broad categories, depending on the gestational age (GA) at delivery: placental PE (PLPE or Type 1; GA < 34 weeks) and maternal PE (MTPE or Type 2; GA ≥ 34 weeks). While the first sub-type results from the poor

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development of early placenta, the second category is associated with an abnormal maternal response to pregnancy (Maynard et al., 2003).

Neuropeptide Y (NPY) is a sympathetic neurotransmitter co-released with norepinephrine. Changes in circulating NPY levels are associated with stress and depend on intensity of sympathetic nervous system (SNS) activation and its duration (Heilig, 2004; Zukowska-Grojec, 1995). Interestingly, such stress-induced increase in NPY levels is observed in platelet rich plasma (PRP), but not platelet poor plasma (PPP), suggesting its accumulation in platelets, rather than elevated levels of free circulating peptide (Kuo et al., 2007; Li et al., 2005; Li et al., 2011; Najafi et al., 2013; Tilan et al., 2013). Increases in NPY levels appear to be temporally associated with elevated blood pressure and vasoconstriction (Hashim and Tadepalli, 1995; Hauser et al., 1996; Zukowska-Grojec, 1995).

Cardiovascular effects of NPY are mediated by several NPY receptor subtypes. NPY Y_1 receptor (Y1R) is the predominant receptor expressed on vascular smooth muscle cells, responsible for vasoconstrictive effects of NPY (Modin et al., 1999; Racchi et al., 1997). In contrast, Y2R is expressed on endothelial cells and is the main angiogenic receptor of NPY (Ekstrand et al., 2003; Lee et al., 2003). The balance between Y1R- and Y2R-mediated activity of NPY is regulated by dipeptidyl peptidase IV (DPPIV), a membrane protease expressed in variety of cells, including endothelium and immune cells (Kitlinska et al., 2003). DPPIV cleaves NPY to its shorter form, $NPY_{3\text{-}36}$, which does not bind Y1R, but preserves its activity at Y2R (Mentlein et al., 1993). Thus, depending on the local DPPIV activity and ratio between Y1R and Y2R expression, NPY can act either as a vasoconstrictor or an angiogenic factor (Kitlinska et al., 2003).

Since the number of adrenergic nerve fibers in the uterine artery decreases in pregnancy, while the presence of NPY-containing nerves increases, it has been proposed that NPY takes over the role of the main uterine vasoconstrictor during pregnancy (Mione et al., 1990). Preliminary data on a small number of subjects indicated increased plasma concentrations of NPY in women with eclampsia and PE, as compared to normotensive pregnant women (Khatun et al., 2000). Moreover, systemic NPY concentrations in PE women have been shown to correlate with mean arterial pressure (MAP) before delivery (Kokot et al., 1999). The increased MAP found in this condition is consistent with hyperactivity of the SNS and increased plasma levels of NPY, which result in long-lasting vasospasms and hypertension (Schobel et al., 1996). In line with this, plasma NPY concentrations in PE women returned almost to reference values of normotensive nonpregnant women few days after delivery, showing no correlation between MAP and plasma NPY levels. In this context, the aim of this study was to extend the above finding by testing the levels of NPY in different sub-types of PE, as well as identifying the source of the peptide.

2. Materials and methods

2.1. Human subjects

The Institutional Review Board at the Georgetown University Hospital (GUH) approved the study protocol. Patients investigated were in the third trimester of pregnancy admitted to GUH from January 2004 until February 2007. The study enrolled 114 subjects either in the normal pregnancy (NP) group (n = 57) or in the PE group (n = 57) after being diagnosed by their physicians and interviewed by investigator (Table 1). Patients with NP had no history of any chronic disease or pregnancy related complications. PE was characterized by hypertension (BP > 140/90 mm Hg) and proteinuria (protein excretion >300 mg/24 h or >1 + protein by dipstick). The PE group was then classified as PLPE (n = 28) when gestational age (GA) at delivery was <34 weeks or MTPE (n = 29) when GA at delivery was ≥34 weeks. Appropriate informed consent was obtained from each participant.

2.2. Exclusion criteria

Women with fever ≥38.3 °C, ongoing bacterial infection (positive blood cultures, clinical suspicion of localized source of infection, or new need for antibiotics during the last 24 h), or an operative blood loss of >800 ml were excluded from the study. Subjects who agreed to participate in the study were assigned a code number to maintain confidentiality.

2.3. Blood samples

10 ml samples of venous blood were collected from patients in the third trimester of pregnancy, after admission and prior to delivery. Blood samples were kept on ice and processed within 30 min after phlebotomy in order to obtain serum (tubes with no anticoagulant) and plasma (EDTA tubes). Blood for serum was allowed to clot and then centrifuged at 2000 rpm for 25 min. PRP was obtained by centrifugation of EDTA-treated blood at 850 rpm for 5 min. PRP was then collected to a

Table 1
Characteristics of the studied population.

		Normal pregnancy	Placental preeclampsia	Maternal preeclampsia
Maternal age at delivery (years)		34 ± 5	31 ± 7	32 ± 7
Parity	Primipara	23.6%	48.3%	51.9%
	Multipara	76.4%	51.7%	48.1%
Number of offspring	Single	96.4%	40.7%	72.4%
	Multiple	3.6%	59.3%	27.6%
Medications	Anti-Hypertensive	0	74.1%	31%
	$MgSO_4$	0	85.7%	65.5%
Type of delivery	Natural vaginal	49.2%	10.7%	28.6%
	Cesarean section	50.8%	89.3%	71.4%
Gestational age at delivery (weeks)		38 ± 1,3	30 ± 2,5	36 ± 1,6
Birth weight (g)		3430 ± 532	1500 ± 520	2731 ± 556
Proteinuria (mg/24 h)		<300[b]	1682.9 ± 255.6[a]	1938.7 ± 221.1[a]
Creatinine clearance (ml/min)		70–100[b]	181.3 ± 92.9[a]	141.2 ± 99.8[a]
Serum creatinine (mg/dl)		0.6–1.2[b]	0.7 ± 0.16[a]	1.6 ± 0.2[a]
Uric acid (mg/dl)		2.1–7.9[b]	6.8 ± 1.4[a]	6.7 ± 0.1[a]
Platelets (100,000/ml)		1.44–3.87[b] 2.2 ± 0.6[a]	1.8 ± 0.4[a]	1.7 ± 0.6[a]
LDL (IU/l)		313–618[b]	228.5 ± 49.9[a]	249 ± 153.1[a]
SOGT (IU/l)		<40[b]	52.8 ± 25.3[a]	108.8 ± 131.1[a]
SGTP (IU/l)		<40[b]	50.5 ± 40[a]	83 ± 100.6[a]
Total number of patients		57	28	29

Data was expressed as the mean ± SEM and absolute value.
[a] Data analyzed correspond to subjects that had blood samples collected and assayed for serum NPY and sFlt-1.
[b] Correspond to mean values in normal patients.