Adrenomedullin, a New Vasoactive Peptide, Is Increased in Preeclampsia

Romolo Di Iorio, Emanuela Marinoni, Claudio Letizia, Piero Alò, Barbara Villaccio, Ermelando V. Cosmi

Abstract—Adrenomedullin is a novel peptide that elicits a long-lasting vasorelaxant activity. Recently, we found high concentrations of adrenomedullin in maternal and umbilical cord plasma and in amniotic fluid in full-term human pregnancy, indicating a role of this peptide during gestation. To investigate the possibility that adrenomedullin is involved in the pathophysiology of preeclampsia, we measured its concentration in maternal and fetoplacental compartments. We studied 12 normotensive nonpregnant women, 13 hypertensive nonpregnant subjects, 29 patients with preeclampsia, and 30 normotensive pregnant women. In all patients, plasma was collected from the cubital vein, and amniotic fluid samples were obtained by transabdominal amniocentesis or at elective cesarean section. Plasma samples from umbilical vein and placental tissues were collected at delivery. Adrenomedullin was assayed on plasma and amniotic fluid samples using a specific radioimmunoassay, and its localization and distribution on placent al sections was determined by immunohistochemistry. Adrenomedullin concentrations were higher in hypertensive than in normotensive nonpregnant patients. Pregnant women had higher adrenomedullin levels than nonpregnant subjects, although maternal plasma adrenomedullin concentrations did not differ between normal pregnant and preeclamptic women. Preeclamptic patients showed higher concentrations (P<0.01) than normotensive pregnant women of adrenomedullin in amniotic fluid (252±29 versus 112±10 fmol/µmol creatinine) and umbilical vein plasma (18.1±2.1 versus 8.5±1.1 fmol/mL). Increased local production of adrenomedullin is associated with preeclampsia. The fetus seems to be responsible for the higher levels of this hormone. Increased adrenomedullin concentrations may be necessary to maintain placental vascular resistance and/or fetal circulation at a physiological level. (Hypertension. 1998;32:758-763.)

Key Words: adrenomedullin • preeclampsia • placenta • amniotic fluid • plasma

Preeclampsia is a serious and common complication of pregnancy, and despite several research efforts, its pathogenesis is still unclear. It has been suggested that failure of trophoblastic invasion of the maternal placental bed may be responsible for impaired placental perfusion, causing the release of cytotoxic factors that cause maternal endothelial damage and dysfunction in a variety of organs.1

During normal pregnancy, important physiological adaptations occur in the mother that ensure an adequate blood supply to the fetus. Vascular resistance, mean arterial pressure, and sensitivity to endogenous constrictors are reduced, whereas cardiac output, heart rate, and blood volume are increased.2 This allows the maintenance of the placent al vasculature in a state of near-maximal dilatation. Failure to achieve these adaptations may result in the reduced fetoplacental perfusion that develops during disease states such as preeclampsia and intrauterine growth retardation. Because placenta lacks autonomic innervation, uteroplacental perfusion is regulated mainly by systemic blood pressure changes through the action of both circulating and locally released vasoactive agents.3 The role of endothelial cells in the modulation of vascular tone has been extensively demonstrated, and abnormal endothelial function in preeclampsia could contribute to an increase in peripheral resistance.4

Adrenomedullin is a novel hypotensive peptide first isolated from human pheochromocytoma eliciting a long-lasting vasorelaxant action.5 Adrenomedullin immunoreactivity and mRNA have been demonstrated in a number of human and animal tissues, including rat uterus.6,7 Cultured endothelial cells secrete adrenomedullin and possess specific receptors for this peptide.8,9 It has been demonstrated that adrenomedullin exerts a natriuretic action on kidneys and peripheral vasculature to control fluid and electrolyte homeostasis10,11 and affects angiotensin II secretion. Recently it has been reported that patients with hypertension show higher levels of plasma adrenomedullin compared with normotensive control subjects, supporting the hypothesis that adrenomedullin could participate in the physiological regulation of blood pressure and vascular homeostasis.12 We recently have found high concentrations of adrenomedullin in full-term pregnancy13.;15
and mRNA for adrenomedullin has been detected in fetal membranes, suggesting a potential role of this peptide in human reproduction.

In the present study, we investigated the possibility that adrenomedullin may be involved in the pathophysiology of preeclampsia and evaluated its concentrations and localization in maternal and fetoplacental compartments.

Methods

Patients

We examined 4 groups of women: (1) 12 normotensive, healthy nonpregnant women (aged 23 to 36 years), (2) 13 hypertensive (WHO stage I) nonpregnant women (aged 25 to 41 years), (3) 29 pregnant women with preeclampsia at 25 to 38 weeks of gestation (aged 21 to 37 years), and (4) 30 normotensive patients (aged 20 to 38 years) matched for gestational age. Preeclampsia was defined as elevated blood pressure (≥140/90 mm Hg) in at least 4 measurements taken 6 hours apart, in association with proteinuria (>0.3 g/d), appearing at a gestational age of >20 weeks. Patients with intrauterine growth retardation associated with hypertension were excluded from the study, as were pregnant women with preexisting hypertension, diabetes, or multiple gestation. None of the pregnant women were in labor at the time of study.

In all subjects, plasma was collected from the cubital vein; in pregnant women, we also obtained amniotic fluid samples by transabdominal amniocentesis or at elective cesarean section. In some patients, umbilical cord plasma samples and placenta specimens were collected at delivery (preeclamptic, n = 11; normotensive, n = 13).

This study was approved by the local ethics committee, and informed consent was obtained from all participants.

Sample Collection

Samples of amniotic fluid were collected by transabdominal amniocentesis or by transuterine amniocentesis at the time of elective cesarean section. In preeclamptic patients, the indication for amniocentesis was fetal lung maturity assessment (16 cases). In the control group, amniocentesis was performed to exclude a fetal infection by cytomegalovirus (polymerase chain reaction technique) suspected on the basis of maternal serology (11 cases). In these patients, prenatal diagnosis and neonatal outcome were negative for the suspected infection. None of the patients experienced uterine contractility before cesarean section. Indications for elective cesarean section in the normotensive group included breech presentation, previous cesarean section, premature rupture of membranes in absence of labor, placenta previa, and maternal cardiac disease. In patients undergoing transabdominal amniocentesis, the initial 3 mL of amniotic fluid was collected and processed. In women undergoing elective cesarean section, samples were collected by transuterine amniocentesis at the time of surgery. Briefly, amniotic fluid samples were immediately centrifuged after collection at 1800g for 15 minutes at 4°C; the supernatants were divided into aliquots and stored at −80°C until assayed.

In pregnant subjects, maternal blood samples were drawn from the cubital vein at the time of amniotic fluid sampling and just before the induction of anesthesia in patients undergoing cesarean section. In 7 preeclamptic patients, blood samples were also collected 48 hours after delivery. In nonpregnant women, blood samples were collected in the first half of the ovarian cycle, and none of the subjects were using oral contraceptives.

At delivery, the umbilical cord was clamped before any signs of breathing were seen; blood was drawn from the umbilical vein. Blood samples, anticoagulated with EDTA and aprotinin, were kept on ice until centrifugation and then were stored at −80°C. Within 10 minutes from delivery, placetas (beginning at 5 cm from the placental disk) were immediately collected and processed for immunohistochemistry.

Adrenomedullin Determination

Plasma and amniotic fluid adrenomedullin concentration was measured after extraction and purification. Briefly, 2 mL of sample was applied to conditioned Sep-Pak C18 columns (Millipore Corp, Waters Chromatography), and the column was sequentially washed with 5 mL of isotonic saline, 5 mL of 0.1% trifluoroacetic acid, and 5 mL of 20% acetonitrile in 0.1% trifluoroacetic acid. The absorbed material was eluted with 4 mL of 50% acetonitrile, and the eluate was lyophilized. After lyophilization, samples were dissolved in 50 mmol/L phosphate buffer (pH 7.4), and adrenomedullin was measured in plasma and amniotic fluid by radioimmunoassay using a commercial kit (Phoenix Pharmaceuticals Inc) with rabbit polyclonal antibody raised against human adrenomedullin 1-52. The antibody cross-reacts 100% with human adrenomedullin; no cross-reactivity was reported with rat adrenomedullin, amylina, calcitonin gene-related peptide, endothelin-1, or α-atrial natriuretic peptide. The intra- and interassay coefficients of variance were 5.1% and 12.0%, respectively.

Immunohistochemistry

Specimens of placental tissue collected at delivery were fixed in 4% paraformaldehyde-0.2% gluteraldehyde, washed, and embedded in paraffin. The presence of adrenomedullin was determined by immunohistochemistry on 5-μm paraffin sections processed as reported.
previously. The sections were stained using the avidin-biotin-peroxidase technique (Vector ABC, Vector Laboratories) and incubated with polyclonal antibody raised in rabbits against purified human adrenomedullin 1-52 (Peninsula Laboratories Inc) at a dilution of 1:600. Negative control tests were conducted on placental tissue incubated with either nonimmune rabbit serum, antibody dilution buffer, or the primary antibody preabsorbed with an excess of human adrenomedullin (1 μmol/L). The number of positive cells was quantified using a quantitative system (field 0.175 mm\(^2\) at \(\times250\) magnification). Ten randomly selected fields were independently counted by 3 different examiners (R. Di I., E.M., P.A.) by visual examination, and the proportion of stained cells was expressed as a percentage of the total cells (stained and unstained). Cells were considered to be positively stained when a brown granular staining of the cytoplasm was revealed at low-power magnification (×10).

Statistics
Adrenomedullin concentrations are expressed as mean±SEM. Statistical analysis was performed with the determination of Spearman rank-order correlation and comparison between groups by Kruskal-Wallis 1-way ANOVA (Dunn’s method) for adrenomedullin concentrations in maternal plasma. The statistical analysis involved the paired t test of adrenomedullin values between the predelivery and postdelivery samples in preeclamptic patients. The Mann-Whitney U test was used for the statistical analysis of adrenomedullin levels in amniotic fluid and umbilical vein plasma between normotensive and preeclamptic women, since data were not normally distributed. To compare proportions of cells stained positive for adrenomedullin, Fisher’s exact test was used. Clinical characteristics of women are expressed as mean±SD and were compared by Student’s t test for unpaired data. Statistical significance was set at \(P<0.05\).

Results
Characteristics of the study subjects are reported in the Table. Because there were fewer umbilical blood and placental tissue samples than maternal blood and amniotic fluid samples, the patient details have been separated according to different components of the study. In the maternal blood and amniotic fluid group, there was no significant difference in gestational age at sampling between normotensive and preeclamptic women; however, the mean gestational age at delivery was significantly lower in the preeclamptic group. In the umbilical blood and placental tissues group, gestational age at sampling corresponded obviously to the gestational age at delivery, and there was no significant difference between patient groups.

Nonpregnant women with hypertension had significantly \((P<0.01)\) higher plasma adrenomedullin concentrations (3.5±0.4 fmol/mL) compared with normotensive nonpregnant women (1.7±0.2 fmol/mL; Figure 1). Adrenomedullin levels in normotensive pregnant patients (10.4±0.9 fmol/mL) were 5-fold higher than those detected in normotensive nonpregnant women. Although in preeclamptic patients plasma adrenomedullin concentrations (11.3±0.9 fmol/mL)
were higher compared with those in normotensive pregnant women, this difference was not statistically significant. Also, when adjusted for maternal serum creatinine, adrenomedullin levels did not differ between normotensive and hypertensive patients, either pregnant or nonpregnant. After 48 hours from delivery in preeclamptic women, plasma adrenomedullin concentrations (6.2±1.5 fmol/mL) were significantly \((P<0.05)\) reduced compared with predelivery values. No correlations were found between adrenomedullin plasma concentrations and gestational age in either normotensive or hypertensive pregnant women. Preeclamptic patients showed higher concentrations \((P<0.01)\) of adrenomedullin than normotensive pregnant patients in umbilical vein plasma (18.1±2.1 versus 8.5±1.1 fmol/mL) and in amniotic fluid (25.6±1.4 versus 17.6±1.4 fmol/mL). To ensure that differences between groups were not due to a different rate of fetal urine production, the amniotic fluid measurements of adrenomedullin were normalized for creatinine as reported\(^6\); after normalization, amniotic fluid adrenomedullin levels were significantly \((P<0.01)\) higher in preeclamptic patients (252±29 versus 112±10 fmol/μmol creatinine; Figure 2).

No correlation was found between amniotic fluid or umbilical plasma adrenomedullin values and gestational age, placental weight, or birth weight in the normotensive and preeclamptic groups.

In placenta, adrenomedullin staining was localized primarily on the extravillous trophoblast cells, as confirmed using cytokeratin in consecutive sections (Figure 3A and 3B), and in scattered areas of syncytiotrophoblast (Figure 3E), although in most of the villi these cells appeared negative. Endothelial cells in the chorionic plate and in the primary villi vessels stained for adrenomedullin. The intensity and percent-

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Figure 3. Immunohistochemical staining of adrenomedullin in normotensive (B and E) and preeclamptic (C and F) placentas. Panel A shows cytokeratin staining (1:1000) in consecutive sections. Positive immunostaining is localized in the extravillous trophoblast cells (t) in both placentas (B and C) as confirmed by cytokeratin (A). Scattered areas of syncytiotrophoblast cells (s) of villi stained for adrenomedullin and immunostaining was localized in endothelial cells (e) of villi and placental basal plate (E and F). No differences were detected in the intensity of immunostaining and the proportion of positive cells between groups. Preabsorption of primary antibody with synthetic peptide (1 μmol/L) shows no immunostaining (D). Magnification ×400.
age of cells stained for adrenomedullin in preeclamptic placenta (Figure 3C and 3F) did not differ from those in sections obtained from normotensive pregnancies. All the tissues examined showed the same immunoreactivity pattern, and no staining was found in negative controls (Figure 3D).

Discussion

This study confirms our previous report on elevated concentrations of adrenomedullin in pregnant women compared with nonpregnant women.\textsuperscript{13} We also found that hypertensive disorder is associated with higher plasma adrenomedullin concentrations than in normotensive patients, as reported by others.\textsuperscript{12} Although maternal adrenomedullin concentrations did not differ significantly between preeclamptic and normal pregnant women, adrenomedullin levels were increased in amniotic fluid and umbilical vein blood collected from preeclamptic pregnant women. In these patients, 48 hours after delivery, adrenomedullin concentrations decreased significantly at levels corresponding to those found postpartum after delivery, adrenomedullin concentrations decreased significantly.

Recent studies have shown that the vasodilatation induced by adrenomedullin is mediated by the release of nitric oxide (NO)\textsuperscript{17} and that NO is necessary to maintain an adequate blood flow through the uteroplacental circulation.\textsuperscript{18} Moreover, adrenomedullin has been demonstrated to inhibit thrombin- and platelet-derived growth factor–induced endothelin-1 (ET-1) production through a cAMP-dependent process.\textsuperscript{19} In the placenta, ET-1 is localized in syncytiotrophoblast and endothelial cells of villi,\textsuperscript{20} and there is evidence that this peptide has a regulatory role in placental circulation.\textsuperscript{15}

The mechanism for increased local production of adrenomedullin in preeclampsia is not known. Amniotic fluid and umbilical vein adrenomedullin concentrations may reflect either fetal or placental production. Adrenomedullin is localized in extravillous trophoblast and endothelial cells of primary villi, suggesting that placenta is a site of production or binding during pregnancy. Although immunohistochemical analysis is not a quantitative technique, we failed to demonstrate any significant differences in either the percentage and intensity of adrenomedullin staining between preeclamptic and normotensive placentas. This finding could indicate that increased adrenomedullin levels in fetoplacental compartments of preeclamptic pregnant women are not derived from the placenta. Alternatively, increased adrenomedullin concentrations in preeclampsia can be explained on the basis of an active secretion of this peptide by the fetus. Actually, endothelial cells from the umbilical vein secrete adrenomedullin in culture systems\textsuperscript{8} and possess specific adrenomedullin receptors.\textsuperscript{9} Furthermore, adrenomedullin has been implicated in the regulation of endothelin secretion. Adrenomedullin inhibits the secretion of ACTH,\textsuperscript{21} aldosterone,\textsuperscript{22} and insulin,\textsuperscript{23} and its secretion is stimulated by thyroid hormones, progesterone, and dexamethasone.\textsuperscript{24} It has been reported that fetuses from preeclamptic women have higher concentrations of corticotropin-releasing hormone, ACTH, and cortisol.\textsuperscript{25,26} Thus, it is possible that adrenomedullin secretion is modulated by these factors and may be involved in the regulation of fetal hypothalamus-pituitary-adrenal gland response to uteroplacental insufficiency.

We can only speculate on the significance of increased adrenomedullin levels found in preeclampsia. Adrenomedullin secreted by the fetus through amniotic fluid and umbilical vein blood may exert its action on placental cells, inducing the release or inhibition of other vasoactive peptide output, such as NO or ET-1, thus participating in the regulation of vascular tone in uteroplacental and fetal circulation. In preeclampsia, we reported that amniotic fluid concentrations of ET-1\textsuperscript{15} and NO\textsuperscript{7} are increased compared with those in normotensive pregnancies, and increased NO levels are necessary to maintain an adequate blood flow through the placenta.\textsuperscript{18,28} We speculate that in preeclampsia, increased local production of adrenomedullin may compensate for the increased synthesis and release from the injured endothelium of other vasoactive substances such as thromboxane A\textsubscript{2} and ET-1, which act as vasoconstrictors on placental vasculature. Thus, increased adrenomedullin concentrations may be necessary to maintain placental vascular resistance and/or fetal circulation at a physiological level. Alternatively, this new vasoactive peptide may have a preeminent role in the regulation of fetal response to a compromised intrauterine environment, acting on fetal endocrine secretion.

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References

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