Effect of Adrenomedullin on Placental Arteries in Normal and Preeclamptic Pregnancies

Sandra Jerat, Donald W. Morrish, Sandra T. Davidge, Susan Kaufman

Abstract—Adrenomedullin is a potent vasodilatory peptide with plasma levels that increase during pregnancy. Although fetoplacental adrenomedullin levels are reported to increase in preeclampsia, maternal plasma levels may be elevated or decreased, or they may resemble those in normal pregnancy. In other hypertensive conditions, adrenomedullin increases. Therefore, we hypothesized that maternal plasma adrenomedullin levels would be higher in hypertensive pregnancies than in normotensive pregnancies and that the higher placental resistance found in preeclamptic pregnancies results from blunted activity of adrenomedullin on the vasculature. Adrenomedullin concentrations in plasma from women with normotensive pregnancies, gestational hypertension, and preeclampsia were determined by radioimmunoassay. Stem villous arteries from normotensive and preeclamptic placentas were dissected and mounted on a wire myograph system. Arteries were first preconstricted to 80% of their maximum constriction with U46619, a thromboxane A2 mimetic, and exposed to cumulative doses of adrenomedullin (1×10⁻⁹ to 3×10⁻⁷ mol/L). Contrary to our hypothesis, there were no significant differences in maternal plasma adrenomedullin levels among patients with normal pregnancies, gestational hypertension, and preeclampsia. Adrenomedullin significantly relaxed arteries from both normal and preeclamptic placentas, but there was no significant difference between the 2 groups. During normal pregnancy, adrenomedullin may contribute to the low placental vascular resistance. This pathway appears to be intact in preeclampsia. We conclude that the increased placental vascular resistance observed in preeclampsia is due neither to reduced adrenomedullin secretion nor to an attenuated vascular responsiveness. Moreover, unlike other hypertensive disorders, there is no compensatory rise in circulating adrenomedullin levels. (Hypertension. 2001;37:227-231.)

Key Words: adrenomedullin ■ preeclampsia ■ arteries ■ pregnancy

Adrenomedullin (ADM) is a 52–amino acid peptide originally discovered in human pheochromocytoma tissue,¹ but it has since been identified in numerous other normal tissues. Among the characteristic actions of ADM are reduction in blood pressure, natriuresis, and diuresis.²⁻³ ADM is secreted from both endothelial cells and vascular smooth muscle cells.⁴ ADM receptors, specific and nonspecific (calcitonin gene–related peptide), are present on endothelial cells and vascular smooth muscle cells.⁵⁻⁷ Depending on the vascular bed and phenotype of the receptive cell, the mechanism of action of ADM may vary⁸; ADM has been shown to exert its effects via stimulation of the NO-cGMP pathway⁹,¹⁰ and activation of adenylyl cyclase–cAMP⁵ and through potassium channel activation.⁹ Because of the increased levels seen in several hypertensive disorders, a potential protective antihypertensive role of ADM has been suggested.¹¹⁻¹⁴

Pregnancy is characterized by a decrease in mean arterial blood pressure despite an increase in blood volume and cardiac output of 40% to 50%.¹⁵ Because ADM is a potent vasodilator, it has been investigated for its potential role in gestation. The concentration of ADM in maternal plasma increases during pregnancy in the human.¹⁶⁻¹⁷ Furthermore, fetoplacental tissues appear to be an additional site of synthesis of ADM during pregnancy.¹⁸ The role of ADM in the pathophysiology of preeclampsia is complex. Maternal plasma levels of ADM in preeclamptic pregnancies compared with uncomplicated pregnancies are reported to be either increased,¹⁹ decreased,²⁰ or the same,²¹,²² despite the fact that ADM levels in amniotic fluid and umbilical vein plasma are reported to be significantly increased in preeclampsia.²¹

Because the placenta is devoid of any autonomic innervation,²³ locally produced factors are essential in maintaining the low vascular resistance present within the placental circulation. A paracrine or autocrine mode of action of ADM has been inferred from the elevated concentrations found in fetoplacental tissues.¹⁸ We hypothesized that maternal plasma ADM levels should, as in other hypertensive disorders,¹¹,¹²,¹⁴ be higher in hypertensive pregnancies than in normotensive pregnancies and that the higher placental resistance found in preeclamptic pregnancies results not from a lack of locally produced ADM but from blunted activity of ADM on the...
vasculature. Therefore, we sought to evaluate maternal plasma ADM levels and the effect of ADM on placental arteries in both preeclamptic and normal pregnancies.

Methods

Experiment A: Maternal Plasma Levels of ADM

Patient Population

The present study was approved by the institutional ethics review board. Preeclampsia was defined by using the criteria of hypertension and proteinuria (dipstick ≥1+). Hypertension was defined as ≥140/90 mm Hg on 2 occasions at least 6 hours apart and occurring after the 20th week of gestation. Gestational hypertension (GH) was defined by using the above-mentioned criteria for hypertension, but with no proteinuria. Women with uncomplicated pregnancies were not corrected for peptide recovery.

Sample Collection

After informed consent was obtained, blood samples were collected on ice into tubes containing EDTA plus 500 KIU aprotinin per 5-mL tube. Women had a blood sample drawn on 1 occasion within 1 of the 3 time intervals. Samples were centrifuged within 30 minutes of collection. Plasma was separated and stored at −70°C until it was ready for extraction.

Plasma Extraction

Plasma samples (1 mL) were extracted as described by Lewis et al. Briefly, plasma was mixed with an equal volume of phosphate alkaline–treated casein buffer. Sep-Pak C-18 columns (Waters Corp) were preequilibrated with 5 mL of methanol and 10 mL of 0.9% saline. The plasma-buffer mixture was added, and the columns were washed with 5 mL of 0.9% saline. ADM was eluted with 2 mL of 80% isopropanol/0.013 mol/L HCl into a tube containing 10 μL of 1% Triton. The eluate was dried under nitrogen, and the extract was stored at −70°C until radioimmunoassay (RIA). Extraction efficiency was measured with the addition of a known amount of unlabeled ADM to plasma with a known amount of endogenous ADM. Recovery was calculated by comparing measured ADM levels with a control of RIA buffer with the same amount of ADM added without extraction. Recoveries of unlabeled ADM in this extraction procedure were 85% in normotensive pregnant plasma, 81% in preeclamptic plasma, and 72% in nonpregnant plasma. There was no significant difference in recoveries between these groups.

Radioimmunoassay

Before analysis, plasma extracts were reconstituted with 250 μL of RIA buffer. Each sample was assayed in duplicate for human ADM (RIA, Phoenix Pharmaceuticals). Plasma samples from all 3 groups were analyzed in any given assay. The intra-assay and interassay coefficients of variance were 7.5% and 10.0%, respectively. Data were not corrected for peptide recovery.

Solutions

The phosphate alkaline–treated casein buffer contained 0.05 mol/L phosphate buffer (pH 7.4), 0.1% alkali-treated casein, 0.1% Triton X-100, 0.1% sodium EDTA, and 0.2% sodium azide. Alkali-treated casein was prepared according to a method described previously. The RIA buffer contained 19 mmol/L sodium phosphate, 81 mmol/L dibasic sodium phosphate, 0.05 mol/L sodium chloride, 0.1% BSA, and 0.01% sodium azide.

Statistical Analysis

Data were analyzed by using the Mann-Whitney rank sum test. Significance was accepted at a value of P<0.05.

Experiment B: ADM-Induced Relaxation in Placental Arteries

Tissue Preparation

Placentas were obtained after vaginal delivery or caesarian section from both normotensive and preeclamptic pregnancies. It has been shown that the mode of delivery has no effect on the responsiveness of placental vessels. Immediately after removal of the placenta, a piece was cut from a macroscopically normal cotyledon and placed in cold HEPES–physiological salt solution (PSS). Small stem villous arteries, averaging 300 μm in diameter and 2 mm in length, were then dissected from the placenta to remove surrounding trophoblastic and connective tissue. The stem villous artery was chosen because it is the major site of resistance in the placenta; the umbilical and chorionic plate vessels do not contribute greatly to vascular resistance. The isolated arteries were then cut into rings and mounted on a wire myograph system. The arteries were bathed in HEPES-PSS at 37°C and at pH 7.4 for 30 minutes before any manipulation and throughout the duration of the experiment. HEPES-PSS maintains an accurate pH and partial pressure of oxygen similar to air. The passive-tension internal circumference measurements were then determined. Briefly, the diameter of the artery was progressively increased in stepwise increments while the force generated was recorded. The Laplace relationship was used to estimate transmural pressure. The stem villous arteries were then set to 90% of the internal circumference they would have had when relaxed under a normal physiological transmural pressure of ~40 mm Hg. Arteries were then allowed to equilibrate for 30 minutes. At the end of each experiment, the viability of the arteries was tested by use of a potassium chloride–depolarizing solution.

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TABLE 1. Characteristics of Patient Groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Normal Pregnancy (n=96)</th>
<th>GH (n=42)</th>
<th>Preeclampsia (n=37)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age, y</td>
<td>30.0±0.5</td>
<td>31.5±1.0</td>
<td>28.9±0.9</td>
</tr>
<tr>
<td>Term blood pressure, mm Hg</td>
<td>&lt;140/90</td>
<td>145/96±2/2</td>
<td>153/98±2/2</td>
</tr>
<tr>
<td>Parity</td>
<td>1.0±0.1</td>
<td>0.8±0.1</td>
<td>0.5±0.1*</td>
</tr>
<tr>
<td>Graftivity, n</td>
<td>2.6±0.2</td>
<td>2.2±0.2*</td>
<td>2.1±0.2*</td>
</tr>
<tr>
<td>Proteinuria (≥+1 on urine testing)</td>
<td>(0/96)</td>
<td>(0/42)</td>
<td>(0/37)</td>
</tr>
<tr>
<td>Urate, μmol/L</td>
<td>ND</td>
<td>323.7±20.4 (26/42)</td>
<td>334.0±14.5 (27/37)</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>0.36±0.01 (72/96)</td>
<td>0.37±0.01 (38/42)</td>
<td>0.36±0.01 (35/37)</td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>122.3±1.3 (72/96)</td>
<td>123.2±2.4 (38/42)</td>
<td>120.8±2.2 (35/37)</td>
</tr>
</tbody>
</table>

Values are mean±SEM. ND indicates not determined. For values in parentheses, denominator denotes total number of patients, numerator denotes number of patients with available measurements.

*P<0.05 vs normal pregnancy.
Effect of ADM on Normal and Preeclamptic Placental Arteries

Cumulative concentration-response curves were carried out by use of the thromboxane A_2 mimetic U46619 (1×10^{-7} to 1×10^{-5} mol/L) followed by a washout period of 60 minutes. Arteries were then preconstricted with the EC_{80} dose of U46619 (7 minutes), and a cumulative ADM concentration-response curve was completed (1×10^{-9} to 3×10^{-7} mol/L, 5-minute increments). A time-control experiment was performed on a parallel preparation of the same artery without added ADM (time control).

Solutions

The arteries were bathed in HEPES-PSS containing (mmol/L) sodium chloride 142, potassium chloride 4.7, magnesium sulfate 1.17, calcium chloride 1.56, potassium phosphate 1.18, HEPES 10, and glucose 5.5. The potassium chloride–depolarizing solution was made by equimolar replacement of sodium chloride with potassium chloride.

Drugs

U46619 (Cayman Chemical Co) stock solutions were prepared in methyl acetate. ADM (Phoenix Pharmaceuticals) was obtained in lyophilized aliquots so that fresh ADM could be used for each experiment. Further dilutions were made with HEPES-PSS for all drugs.

Statistical Analysis

Repeated-measures ANOVA was used to investigate the relaxation response with ADM or without (time control) in both normal and preeclamptic placentas. A value of P<0.05 was considered significant.

Results

Experiment A

Subjects

Table 1 summarizes the characteristics of the patient groups. Comparisons were made between the GH and preeclamptic group and the normotensive group. Maternal age, hematocrit, and hemoglobin were comparable between groups. Parity was significantly lower in women with preeclampsia (P<0.05).

Maternal Plasma ADM Levels in Normotensive, GH, and Preeclamptic Pregnancies

Comparisons of plasma ADM levels in normotensive, GH, and preeclamptic patients by gestational age grouping are shown in Figure 1. There were no significant differences between groups. Values are expressed as mean±SEM.

Experiment B

Subjects

Table 2 summarizes the characteristics of women with preeclampsia and uncomplicated pregnancies. Maternal age, parity, gravidity, hematocrit, and hemoglobin were comparable between the 2 groups. Gestational age at delivery and infant birth weight were significantly lower in the preeclampsia group (P<0.05). Compared with normotensive pregnant women, women with preeclampsia had significantly higher systolic and diastolic blood pressures (P<0.05).

Effect of U46619 and Potassium Chloride–Depolarizing Solution on Normal and Preeclamptic Placental Arteries

Concentration-dependent constriction occurred in the presence of U46619 (1×10^{-5} to 1×10^{-6} mol/L). There was no significant difference in the EC_{80} values between normal arteries (4.04±0.37 to 4.03±0.86 mol/L) and preeclamptic arteries.

TABLE 2. Characteristics of Patient Groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Normal Pregnancy (n=12)</th>
<th>Preeclampsia (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age, y</td>
<td>26.3±2.1</td>
<td>27.9±1.6</td>
</tr>
<tr>
<td>Term blood pressure, mm Hg</td>
<td>118/71±2/2</td>
<td>166/111±8/4*</td>
</tr>
<tr>
<td>Parity, n</td>
<td>0.8±0.2</td>
<td>0.6±0.4</td>
</tr>
<tr>
<td>Gravidity, n</td>
<td>2.7±0.7</td>
<td>2.0±0.4</td>
</tr>
<tr>
<td>Proteinuria (≥+1 on urine testing)</td>
<td>(0/12)</td>
<td>(9/9)</td>
</tr>
<tr>
<td>Urate, μmol/L</td>
<td>ND</td>
<td>316.1±23.2 (7/9)</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>0.37±0.01 (6/12)</td>
<td>0.37±0.02 (8/9)</td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>124.7±3.7 (6/12)</td>
<td>124.8±5.8</td>
</tr>
<tr>
<td>Gestational age at delivery, wk</td>
<td>38.8±0.4</td>
<td>36.2±0.8*</td>
</tr>
<tr>
<td>Infant birth weight, kg</td>
<td>3.4±0.1</td>
<td>2.6±0.3*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. For values in parentheses, denominator denotes total number of patients, and numerator denotes number of patients with available measurements. *P<0.05 vs normal pregnancy.
Effect of ADM on placental arteries from normotensive and preeclamptic pregnancies. A, Open circles indicate normotensive time-control group (n=12); solid circles, normotensive ADM group (n=12). B, Open triangles indicate preeclamptic time-control group (n=9); solid triangles, preeclamptic ADM group (n=12). Vertical bars delineate SEM. Responses are expressed as a percentage of relaxation from U46619-preconstricted levels.

Effect of ADM on Normal and Preeclamptic Placental Arteries
Concentration-dependent relaxation occurred in the presence of ADM (1×10^{-9} to 3×10^{-7} mol/L) compared with its time control in normal (Figure 2A) and preeclamptic (Figure 2B) placental arteries (P<0.05). For both normal and preeclamptic placental arteries, the effect of treatment on percent relaxation depends on time as indicated by the treatment and time interaction. For normal placental arteries, relaxation with the highest dose of ADM (3×10^{-7} mol/L) was 42±6% compared with its time control (14±5% of U46619-induced contraction, Figure 2A). For preeclamptic placental arteries, relaxation with the highest dose of ADM (3×10^{-7} mol/L) was 26±11% compared with its time control (−1±7% of U46619-induced contraction, Figure 2B). The behavior of these 2-way interaction effects in normal and preeclamptic groups is not statistically different, as shown by the 3-way interaction effect between treatment, time, and group. This was supported by the similarity in the dose-response curves depicted in Figure 2.

Discussion
The present study supports previous findings showing unchanged maternal plasma levels of ADM in normotensive and preeclamptic pregnancies. In addition, we have found no difference in the GH group. The GH subgroup, which has not been previously studied, was included because of the potential difference in etiology compared with preeclampsia.

Other hypertensive diseases, including essential hypertension, renal failure, heart failure, and primary aldosteronism, all show an increase in plasma ADM levels. In these conditions, ADM may serve to compensate for the elevation of blood pressure. In contrast, we have demonstrated that in preeclampsia and GH, there is no compensatory increase in plasma ADM. This could potentially contribute to the hypertension seen in GH and preeclampsia.

Because the placenta is devoid of autonomic innervation, locally produced factors are essential in maintaining the low vascular resistance characteristic of the placental circulation. A paracrine or autocrine mode of action of ADM has been inferred from the elevated concentrations found in fetoplacental tissues. We showed that ADM induces relaxation in the placental circulation in both normotensive and preeclamptic pregnancies. We originally formulated the hypothesis that an attenuated relaxation to ADM would exist in placental arteries from preeclamptic compared with normotensive pregnancies. However, we found no such difference. It has been previously reported that ADM levels are increased in amniotic fluid and umbilical vein plasma in women with preeclampsia compared with women with normotensive pregnancies, suggesting that greater concentrations of ADM are available locally in preeclampsia. Because placental arteries from preeclamptic pregnancies retain their ability to respond to ADM, this could potentially reflect a compensatory mechanism to counteract the increase in vascular resistance characteristic of the condition. It should be noted that we preconstricted the arteries with U46619. It is possible that in the presence of other vasoconstrictor agents, the relaxation response to ADM may differ.

Although we did not find a difference in ADM-induced relaxation between groups, we did find that the tension developed in response to U46619 and potassium chloride–depolarizing solution was significantly less in arteries from preeclamptic pregnancies than from normotensive pregnancies. Therefore, altered vasoconstrictive activity may exist in placentas obtained from women with preeclampsia. A placental lobule perfusion method likewise demonstrated a reduced pressure increase created by U46619 in placentas from women with preeclampsia compared with those with normotensive pregnancies. In addition, the constrictor response elicited in umbilical arteries from women with preeclampsia demonstrated a decreased sensitivity to a potassium chloride–depolarizing solution. The decreased constrictor response to these agents in preeclampsia suggests a compensatory mechanism to counteract the increased resis-
tance seen in this condition. However, no such change has been found in chorionic plate arteries.\textsuperscript{33}

In summary, although ADM levels are elevated in many other hypertensive disorders, we did not find a significant difference in maternal plasma concentrations in either eclampsia or GH compared with normal pregnancy. This lack of a compensatory response to the increased blood pressure may potentially worsen the cardiovascular state of such patients. We have also shown, for the first time, that ADM causes a dose-dependent relaxation of placental arteries. We conclude that ADM-induced vasodilation may contribute to the low vascular resistance seen in normal pregnancy. Furthermore, retention of this vasorelaxant activity in placental arteries derived from preeclamptic pregnancies may serve to attenuate the significant increase in placental vascular resistance associated with this condition. Studies are ongoing to investigate the mechanisms underlying the vasorelaxation caused by ADM in the placenta.

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References


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