Eclamptic Plasma Stimulates Norepinephrine Release in Cultured Sympathetic Nerve

Selina Khatun, Naohiro Kanayama, Eiji Sato, Hossain M. Belayet, Takao Kobayashi, Toshihiko Terao

Abstract—The purpose of the present study was to evaluate the effect of plasma from eclamptic and preeclamptic patients on cultured sympathetic nerve. Sympathetic neurons from 12- to 14-day-old chick embryos were cultured; the neurons were then stimulated with 50% plasma from eclamptic, preeclamptic, hypertensive, normotensive pregnant, hypertensive, and normotensive nonpregnant women (n=7). Similarly, neurons were individually incubated with mixtures of 50% corresponding plasma with 0.25% bupivacaine or bupivacaine only (n=7). Furthermore, the effects of 1%, 10%, and 50% plasma from eclamptic, preeclamptic, and normotensive pregnant patients (n=7) were also evaluated. Norepinephrine concentrations were measured by high-performance liquid chromatography. Electron microscopic studies of nerve cells were also performed. Stimulation with plasma from eclamptic and preeclamptic women significantly increased norepinephrine concentration (P<0.0001) compared with control. The release of norepinephrine was found to be concentration-dependent. Conversely, norepinephrine secretion was significantly hampered by bupivacaine treatment (P<0.0001). Electron microscopic studies in eclamptic and preeclamptic plasma–stimulated nerve cells showed that perikarya were in close contact with each other and with nerve cell processes. After treatment with bupivacaine, nerve cells were irregular in shape and the cell membranes were demyelinated. These results suggest that eclamptic and preeclamptic plasma has an excitotoxic effect on sympathetic nerve via axoplasmic membrane depolarization, thus increasing norepinephrine secretion that is blocked by bupivacaine. A preeclamptic condition may be improved by depression of sympathetic nerve stimulation. (Hypertension. 1998;31:1343-1349.)

Key Words: eclampsia ■ sympathetic nervous system ■ norepinephrine ■ chick embryo ■ bupivacaine

Experimental studies have provided unequivocal evidence that eclampsia and preeclampsia are states of sympathetic hyperactivity.1,2 NE concentrations in plasma, platelets, and urine are elevated in preeclampsia and eclampsia.1,3–6 NE is a neurotransmitter released from the sympathetic nerve endings, and stimulation of sympathetic nerve facilitates NE secretion.7 Recently, Schoebel et al7 measured sympathetic activity in the peroneal nerve with microneurography and found that the postganglionic action potential was increased in preeclamptic women.

The sympathetic nerve tends to be irritated by various stimulations such as environmental stress, physical stress, mental stress, postural change, and insulin. Stimulation of sympathetic nerve is not only involved in the pathophysiology of preeclampsia but can also be a major cause of preeclampsia.8 Several other studies have suggested previously that preeclamptic women exhibiting heightened sympathetic activity are those with severe disease, with such activity being a secondary response to other factors such as plasma volume contraction.9 There is accumulating evidence for a pathogenic model of preeclampsia, whereby stimulation of sympathetic nerve results in decreases in placental blood flow, which are somehow translated into a multisystem maternal disorder characterized by vasconstriction, platelet activation, and intravascular coagulation, as well as increased capillary permeability. The most popular hypothesis is that the ischemic fetoplacental unit releases factors into the maternal circulation that lead to systemic pathological changes with widespread endothelial cell damage.9,10 For instance, the repeated vasospasm and increased ET-1 levels found in eclampsia and preeclampsia are evidence of endothelial cell disorder in this disease.11,12 Moreover, the action of ET-1 on peripheral postganglionic sympathetic nerves mimics neuronal activity that causes rapid alterations in NE concentrations.13 This attenuation could be reversed by the replacement of lumbar epidural anesthesia, which blocks the abdominal sympathetic nerve, thus improving hypertension and biochemical parameters during preeclamptic labor. In preeclampsia, the vascular changes are acute and transient and react more rapidly to sympathetic blockade.14

Our focus here is to elucidate whether stimulation of the sympathetic nerve with plasma from eclamptic and preeclamptic women could induce NE release in cultured sympathetic neurons.
Diastolic BP, mm Hg 112.0

characteristics of the patients are summarized in Table 1.

preeclampsia. The subjects were comparable with respect to age, that the hypertension found in these patients was not related to diabetes, chronic renal disease, platelet disorder, maternal or fetal cases complicated by essential hypertension, cardiovascular disease, the patients had hypertension before the 20th week of pregnancy. All

Patient age, y 21.6

Characteristic

Nonpregnant Women

Eclampsia

Preeclampsia

Hypertensive Pregnant

Normotensive Pregnant

Hypertensive Nonpregnant

Normotensive Nonpregnant

Eclampsia

Preeclampsia

Hypertensive Pregnant

Normotensive Pregnant

Hypertensive Nonpregnant

Normotensive Nonpregnant

Patient age, y 21.6

Body weight, kg 72.1±6.3

Parity Primipara Primipara Primipara Primipara

Gestational age, wk 35.0±2.2

Systolic BP, mm Hg 166.3±13.0

Diastolic BP, mm Hg 112.0±10.8

Proteinuria, paper stick method + + + +

Values represent mean±SD. BP indicates blood pressure, +, mild to moderate; and ++, moderate to severe.

*P<0.05 compared with normotensive pregnant patients (ANOVA factorial analysis).

Methods

Subjects

This study was approved in April 1995 by the research committees of the institutes concerned, and all studies were performed in accordance with institutional guidelines for human and animal research. Consent was obtained from the patients or the patients' guardians (in the case of eclamptic patients). We investigated eclamptic, preeclamptic, hypertensive, and normotensive pregnant patients (n=7) in the third trimester of pregnancy who were admitted to Dhaka Medical College Hospital, Bangladesh. Normotensive and hypertensive nonpregnant volunteer women (n=7) were also enrolled in this study. All the eclamptic patients essentially met the criteria for preeclampsia and also had at least one episode of eclamptic convulsion either in the hospital or at home. Based on history and clinical findings, there was no other reason for the onset of seizures in the eclamptic patients. Preeclampsia was defined as the development of hypertension with proteinuria induced by pregnancy after the 20th week of gestation. Hypertension was defined as a diastolic blood pressure of at least 90 mm Hg or systolic blood pressure of at least 140 mm Hg, or a rise in the former of at least 15 mm Hg or in the latter of 30 mm Hg over baseline values on two consecutive readings at least 6 hours apart, with blood pressure reverting to normal within 2 months after delivery. Proteinuria was defined as the presence of ≥300 mg protein in a 24-hour urine collection or a protein concentration of ≥1 g/L in at least two random urine specimens collected 6 hours or more apart.¹ None of the patients had hypertension before the 20th week of pregnancy. All cases complicated by essential hypertension, cardiovascular disease, diabetes, chronic renal disease, platelet disorder, maternal or fetal infection, autoimmune disorders, and epilepsy were excluded from this study. All hypertensive pregnant patients suffered from essential hypertension, and none of the patients had proteinuria. This indicates that the hypertension found in these patients was not related to preeclampsia. The subjects were comparable with respect to age, body weight, and duration of gestation. The important clinical characteristics of the patients are summarized in Table 1.

Sample Collection

Blood was drawn in the morning in all except eclamptic patients, for whom it was taken during admission to the emergency department of the hospital. None of the eclamptic patients had received any antihypertensive or anticonvulsant drugs before collection of blood. Peripheral venous blood samples were collected immediately after measurement of blood pressure by Korotkoff first and fifth sounds with the patient in the recumbent position. Plasma samples (prepared from blood collected in EDTA) were studied to avoid the confounding effects of cellular products released into serum during blood coagulation. Briefly, samples were maintained at 26°C for 2 to 4 hours before centrifugation at 2000 g for 20 minutes; they were then portioned into aliquots under sterile conditions and stored at −80°C. Samples obtained in Bangladesh were transported by air in dry ice (at −40°C, within 16 hours) to Hamamatsu University School of Medicine, Japan, where all experiments were performed. There were no significant differences between subject groups in time of storage or time from venipuncture to centrifugation.

Analytical Methods

Platelets in plasma from 1-mL EDTA anticoagulated blood samples were counted with a Coulter model ZM with a 70-μm aperture tube.¹¹ Platelet-rich plasma was prepared by centrifugation at 120g for 10 minutes, separated from the sedimented red blood cells with a plastic pipette, and transferred to a capped polystyrene tube. Platelet counts were done with platelets in isotonic NaCl solution at room temperature. Hematocrit level was determined in these blood samples before plasma was spun off by the microhematocrit method using heparinized capillary tubes (SRL) centrifuged in an MB centrifuge (International Equipment Company). For measurement of ET-1, plasma samples were subjected to a sandwich-type EIA (Takeda Chemical Industries Ltd). ET-1 was extracted from 0.5 mL plasma using Sep-Pak C-18 cartridges (Waters, Millipore Corp). The extracts were dissolved in 250 μL of buffer D and measured with a sandwich EIA. Briefly, microtest plates coated with rabbit anti–ET-1 C-terminal heptapeptide (15–21) antibodies (30821) were incubated at 37°C for 1 hour with 100 μL of standard solutions or plasma samples. After a washing with PBS, the plates were allowed to react at 37°C for 30 minutes with 100 μL horseradish peroxidase–labeled rabbit anti–ET-1 N-terminal loop domain antibody (30846). The plates were washed with PBS, and the bound enzyme activities were measured using o-phenylenediamine as a chromogen. The EIA did not cross-react with big ET-1. The detection limit of ET-1 was 0.4 nmol per well.

Neuron Culture

Sympathetic neurons were obtained from the paravertebral lumbar sympathetic chains of 12- to 14-day-old chick embryos (White Leghorn strain). The culture was done as previously described by Freshney.¹⁶ Briefly, the lumbar sympathetic trunks were separated, and the individual ganglia were then dissected by cutting the trunks between each ganglion, using a stereomicroscope (Zeiss, Stemi 2000). The sympathetic neurons were comparable in size and weight. The ganglia were immersed in a culture nutrient medium on glass coverslips in Petri dishes (35×10 mm, Falcon Becton Dickinson).

### TABLE 1. Characteristics of Eclamptic, Preeclamptic, Hypertensive, Normotensive Pregnant, Hypertensive, and Normotensive Nonpregnant Women

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Eclampsia (n=7)</th>
<th>Preeclampsia (n=7)</th>
<th>Hypertensive Pregnant (n=7)</th>
<th>Normotensive Pregnant (n=7)</th>
<th>Hypertensive Nonpregnant (n=7)</th>
<th>Normotensive Nonpregnant (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient age, y</td>
<td>21.6±2.6</td>
<td>23.0±3.1</td>
<td>24.7±3.8</td>
<td>22.9±3.7</td>
<td>28.9±2.7</td>
<td>24.0±2.7</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>72.1±6.3</td>
<td>70.3±4.3</td>
<td>68.3±3.3</td>
<td>66.1±2.6</td>
<td>62.1±5.2</td>
<td>51.9±3.2</td>
</tr>
<tr>
<td>Parity</td>
<td>Primipara</td>
<td>Primipara</td>
<td>Primipara</td>
<td>Primipara</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Gestational age, wk</td>
<td>35.0±2.2</td>
<td>36.0±2.2</td>
<td>36.4±1.7</td>
<td>36.3±2.4</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>166.3±13.0</td>
<td>163.7±11.4*</td>
<td>146.6±4.1*</td>
<td>113.9±7.8</td>
<td>155.1±9.4*</td>
<td>115.1±11.8</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>112.0±10.8*</td>
<td>102.1±8.1*</td>
<td>94.7±3.0</td>
<td>75.7±4.5</td>
<td>103.3±5.4*</td>
<td>73.4±7.8</td>
</tr>
<tr>
<td>Proteinuria, paper stick method</td>
<td>+ + + +</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

Values represent mean±SD. BP indicates blood pressure, +, mild to moderate; and ++, moderate to severe.

*P<0.05 compared with normotensive pregnant patients (ANOVA factorial analysis).
The media consisted of cockerel plasma (50 µL, Japan Biotest Institute), chick embryonic extract (5 µL), Eagle’s minimal essential medium (45 µL, MEM, Nissui Pharmaceutical Co, Ltd), and nerve growth factor (5 µL, 100ng/mL concentration 7S NGF, Wako). Plasma clots were made of equal parts of heparinized cockerel plasma and chick embryonic extract with Eagle’s minimal essential medium. The dishes were kept in a chamber of an incubator (Astec) at that was gassed with 5% CO₂/95% O₂ and incubated for 24 hours at 37°C.

**Stimulation With Different Percentages of Plasma and Bupivacaine**

After 24 hours of culture, the sympathetic nerves were randomized and incubated for 2 hours with 1%, 10%, and 50% plasma from eclamptic, preeclamptic, hypertensive, and normotensive nonpregnant women. In addition, sympathetic neurons were incubated with mixtures of bupivacaine (0.25%) with individual plasma (50%) from eclamptic, preeclamptic, hypertensive, normotensive pregnant, hypertensive, and normotensive nonpregnant women. Sympathetic nerve stimulation also was conducted with bupivacaine (0.25%) only. Each incubation was performed in duplicate wells on seven separate occasions. The samples were collected and homogenized mixed for NE measurement. Moreover, the NE concentration was also measured in plasma in diluted condition (50% plasma and 50% culture media) from eclamptic, preeclamptic, hypertensive, normotensive pregnant, hypertensive, and normotensive nonpregnant women.

**Measurement of NE Concentration**

The concentrations of NE were measured with the Tosoh automatic catecholamine analyzer HLC-725CA, an HPLC system that uses the specific highly sensitive fluorescent reagent diphenylethylenedia mine (DPE) for catecholamine measurement. This analyzer can measure as little as 0.03 nmol/L of NE. Samples were analyzed in duplicate after the triplicate determination of the standard solution, 1 nmol/L of NE hydrochloride. Plasma concentrations of NE were calculated by comparing the peak heights of samples with those of the standard solution. The standard NE solution and NE concentration in the samples were measured 15 times consecutively to determine the reproducibility of the measured free NE concentration. The reproducibility of the sample’s NE concentration was 4.17±0.041 nmol/L. The intra-assay coefficient variation for free NE was <1.0%.

**Electron Microscopic Study**

The stimulated sympathetic nerve tissues were fixed for electron microscopic study with 2% glutaraldehyde in 0.14 mol/L phosphate buffer (pH 7.4) for 2 hours at 4°C. After a washing with 0.14 mol/L phosphate buffer, the specimens were postfixed with 1% OsO₄ in the same buffer for 2 hours. The specimens were then dehydrated in an ethanol series, and cells were embedded in epoxy resin. The ultrathin sections were cut with an LKB-Ultratome, using a diamond knife, and poststained with lead citrate and uranyl acetate. The microscopic observations and photographs were made using a JEOL JEM 1220 electron microscope at 80 kV.

**Statistical Analysis**

The results are reported as mean±SD. A value of P<0.05 was considered statistically significant. Repeated measure ANOVA was used to determine the difference between percent concentration variables. Factorial ANOVA was used to analyze the changes in NE concentration after different stimulations and to compare the clinical parameters in eclamptic, preeclamptic, hypertensive, normotensive pregnant, hypertensive, and normotensive nonpregnant women.

**Results**

The concentrations of NE after stimulation of cultured sympathetic nerve with 50% plasma from eclamptic, preeclamptic, hypertensive, normotensive pregnant, hypertensive, and normotensive nonpregnant women. The intra-assay coefficient variation for free NE was 4.17±0.041 nmol/L. The intra-assay coefficient variation for free NE was <1.0%.

The concentrations of NE after stimulation of cultured sympathetic nerve were 13.4±1.2 nmol/L, and after stimulation with bupivacaine this concentration decreased to 9.0±0.7 nmol/L. Stimulation with eclamptic and preeclamptic plasma showed a significant increase in NE level compared with control (22.0±1.1 and 19.2±1.3 nmol/L, respectively; P<0.0001). A marked decrease in NE secretion also was observed after treatment with the mixture of plasma and bupivacaine, which was greater than that after stimulation with respective plasma only (16.6±1.6 and 15.5±1.2 nmol/L, respectively; P<0.0001). No significant changes in NE content were found after stimulation with plasma from hypertensive, normotensive pregnant, hypertensive, and normotensive nonpregnant women (14.2±1.2, 13.9±1.3, 13.7±0.9, and 13.4±1.1 nmol/L, respectively) compared with control. Moreover, a significant decrease in NE secretion was also found after individual stimulation with the mixtures of respective plasma and bupivacaine compared with stimulation with corresponding plasma only (9.8±0.8, 9.3±0.9, 9.2±0.9, and 9.0±1.5 nmol/L, respectively; P<0.0001). NE concentrations in diluted plasma from eclamptic, preeclamptic, hypertensive, normotensive pregnant, hypertensive, and normotensive nonpregnant women were found to be 5.7±0.6, 5.3±0.5, 4.9±0.6, 4.8±0.7, 4.7±0.7, and 4.5±0.6 nmol/L, respectively.

The NE concentrations in the cultured sympathetic nerve after stimulation with different percentages of plasma from eclamptic, preeclamptic, hypertensive, normotensive pregnant, hypertensive, and normotensive nonpregnant women and a mixture of 50% corresponding plasma with 0.25% bupivacaine are shown in Figure 1. The NE concentration in the control cultured sympathetic nerve was 13.4±1.2 nmol/L, and after stimulation with bupivacaine this concentration decreased to 9.0±0.7 nmol/L. Stimulation with eclamptic and preeclamptic plasma showed a significant increase in NE level compared with control (22.0±1.1 and 19.2±1.3 nmol/L, respectively; P<0.0001). A marked decrease in NE secretion also was observed after treatment with the mixture of plasma and bupivacaine, which was greater than that after stimulation with respective plasma only (16.6±1.6 and 15.5±1.2 nmol/L respectively; P<0.0001). No significant changes in NE content were found after stimulation with plasma from hypertensive, normotensive pregnant, hypertensive, and normotensive nonpregnant women (14.2±1.2, 13.9±1.3, 13.7±0.9, and 13.4±1.1 nmol/L, respectively) compared with control. Moreover, a significant decrease in NE secretion was also found after individual stimulation with the mixtures of respective plasma and bupivacaine compared with stimulation with corresponding plasma only (9.8±0.8, 9.3±0.9, 9.2±0.9, and 9.0±1.5 nmol/L, respectively; P<0.0001). NE concentrations in diluted plasma from eclamptic, preeclamptic, hypertensive, normotensive pregnant, hypertensive, and normotensive nonpregnant women were found to be 5.7±0.6, 5.3±0.5, 4.9±0.6, 4.8±0.7, 4.7±0.7, and 4.5±0.6 nmol/L, respectively.

![Figure 1](http://hyper.ahajournals.org/ content/1345/i1878-9728-5-1-626/F1.large.jpg)
Eclampsic, preeclamptic, and normotensive pregnant patients are illustrated in Figure 2. Stimulation with 10% and 50% eclamptic and preeclamptic plasma significantly increased NE secretion (P<0.0001) compared with stimulation with 1% plasma. However, the NE concentration was higher after stimulation with eclamptic plasma than with preeclamptic plasma. Furthermore, 50% eclamptic and preeclamptic plasma increased NE secretion to a greater extent than did 10% plasma (P<0.0001). Treatment with 1% plasma from eclamptic and preeclamptic patients and different percentages of plasma from normotensive pregnant patients had no significant effect on NE secretion.

The platelet count, hematocrit level, and ET-1 concentration in eclamptic, preeclamptic, hypertensive, normotensive pregnant, hypertensive, and normotensive nonpregnant women are summarized in Table 2. Platelet count significantly decreased, ET-1 level markedly increased, and severe hemoconcentration was observed in eclamptic (P<0.0001) and preeclamptic (P<0.0001) patients compared with normotensive hypertensive pregnant women. Fewer significant changes in platelet count, hematocrit level, and ET-1 concentration were found in hypertensive pregnant, hypertensive, and normotensive nonpregnant women than in normotensive pregnant patients.

The correlation between NE concentration in the cultured sympathetic nerve after stimulation with eclamptic and preeclamptic plasma and ET-1 level in the plasma of the corresponding patients is shown in Figure 3. A significant positive correlation between NE concentration and ET-1 levels was observed in both cases. The correlation coefficient and probability values were r=0.677, P<0.0001 and r=0.583, P<0.0001, respectively.

The cultured explants of the chick sympathetic nerve consisted of neural and nonneural cells. The electron micrographs of nerve cell histology are shown in Figure 4. The histology of nerve, stimulated by plasma from eclamptic and preeclamptic women, shows that nerve cells were enlarged and irregular in shape (4A and 4B). The perikarya of nerve cells were in close contact with each other and with the nerve cell processes. The nerve fibers were in tightly arranged fascicles between the cells, compared with the control and sham control histology (4C and 4D). Demyelination of the cell membranes and irregular shapes were found in bupivacaine-treated nerve (4E).

**Discussion**

The major findings of the present study are that the NE contents in sympathetic neurons were markedly enhanced when they were stimulated with plasma from eclamptic and preeclamptic women compared with plasma from normotensive pregnant women. It is likely that plasma from eclamptic and preeclamptic women contains various factors that exert a trophic influence on the sympathetic neurons to increase their noradrenergic properties. Complicated pregnancies, such as those involving eclampsia, show marked changes in stimulation of the sympathetic nervous system in the production of

![Graph showing NE concentrations after stimulation of cultured sympathetic nerve with different plasma concentrations.](image)

**Figure 2.** NE concentrations after stimulation of cultured sympathetic nerve with 1%, 10%, and 50% plasma from eclamptic, preeclamptic, and normotensive pregnant women. Stimulation with 10% and 50% eclamptic and preeclamptic plasma significantly increased the NE concentration. The level of NE was markedly greater after stimulation with eclamptic plasma than after preeclamptic stimulation. Incubation with 1% eclamptic plasma, preeclamptic plasma, and different concentrations of normotensive pregnancy plasma could not induce the secretion of NE. Values represent mean±SD, n=7. *P<0.05 vs control.

**Figure 3.** Correlation between NE concentration in the cultured sympathetic nerve (after stimulation with eclamptic and preeclamptic plasma) and ET-1 level in the respective plasma is shown. NE concentration was significantly correlated with ET-1 level in eclamptic (r=0.677, P<0.0001) and preeclamptic (r=0.583, P<0.0001) plasma.

**Table 2. Clinical Parameters of Eclamptic, Preeclamptic, Hypertensive, Normotensive Pregnant, Hypertensive, and Normotensive Nonpregnant Women**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Eclampsia (n=7)</th>
<th>Preeclampsia (n=7)</th>
<th>Hypertensive Pregnant (n=7)</th>
<th>Normotensive Pregnant (n=7)</th>
<th>Hypertensive Nonpregnant (n=7)</th>
<th>Normotensive Nonpregnant (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit, ×10^-2</td>
<td>48.8±4.3*</td>
<td>46.7±6.1*</td>
<td>33.3±4.6</td>
<td>32.3±4.2</td>
<td>30.8±2.5</td>
<td>30.0±3.6</td>
</tr>
<tr>
<td>Platelet count, ×10^9/L</td>
<td>23.1±7.5*</td>
<td>25.6±4.1*</td>
<td>32.4±4.9</td>
<td>33.4±4.8</td>
<td>34.5±4.3</td>
<td>34.1±3.9</td>
</tr>
<tr>
<td>ET-1 levels, pmol/L</td>
<td>2.7±0.5*</td>
<td>2.3±0.5*</td>
<td>0.8±0.3</td>
<td>0.7±0.1</td>
<td>0.6±0.3</td>
<td>0.6±0.2</td>
</tr>
</tbody>
</table>

Values represent mean±SD.

*P<0.05 compared with normotensive pregnant patients (ANOVA factorial analysis).
An earlier report demonstrated that the postganglionic action potential in sympathetic nerve fibers was increased in preeclampsia. Recently, we suggested that sympathetic nerve stimulation is responsible for vasospasm and endothelial injury resulting in preeclampsia-like phenomena. In our present experiment, stimulation with plasma from eclamptic patients showed higher values than plasma from preeclamptic patients. Eclampsia produces more acute stress than pre-eclampsia, resulting in an abnormal stimulation of the sympathetic nervous system. The seizures in the eclamptic condition might accelerate the production of factors that are responsible for increased secretion of NE. The mechanism by which NE secretion is increased is not clearly known. There may be two possibilities: (1) increases in the synthesis of NE in the nerve cell and (2) increases in neuronal release of NE from storage sites. Stimulation of the sympathetic nervous system in eclampsia and preeclampsia may also be a secondary phenomenon.

Previous observations demonstrated that biosynthesis of NE was accelerated by application of biopterin, a cofactor for tyrosine hydroxylase also known as catecholamine biosynthetic enzyme. Kessler found that the membrane depolarization in cultures stimulates the development of noradrenergic traits, such as tyrosine hydroxylase, and increases catecholamine synthesis in sympathetic neurons. It is well known that plasma of eclamptic and preeclamptic women contains high levels of ET-1, insulin, and thrombin. ET-1 and insulin were found to stimulate the sympathetic nerve, and the existence of thrombin receptor in the nerve cells was demonstrated. Although the exact factors responsible for sympathetic nerve stimulation in this study are not clearly understood, such mediators might be candidates. The depolarization effects of ET-1 on peripheral postganglionic sympathetic neurons have been suggested. A significant correlation between the NE and ET-1 concentrations exists in both eclamptic and preeclamptic patients. NE (a neurohormone) and ET-1 (a marker of endothelial injury) are important parameters to determine the status of this disease. Elevated NE and ET-1 levels might increase parallel to the progression of this disease. Hemoconcentration is another confirming factor in eclampsia and preeclampsia. Increased hematocrit concentration and decreased platelet count in eclamptic and preeclamptic women reflect hemoconcentration and intravascular coagulation. One of the early pathophysiological changes in eclampsia and preeclampsia is endothelial cell disorder leading to vasoconstriction, thus...
enhancing capillary permeability and intravascular coagulation. The increase in plasma factors such as ET-1 and hemoconcentration found in our experiment might be responsible for depolarization of sympathetic neurons and thus acceleration of NE secretion. Electron microscopic findings also support the membrane depolarization phenomenon. Clearly, elucidation of the factors of sustained axoplasmic membrane depolarization and the mechanism of NE increment awaits further studies.

A concentration-dependent effect was found after stimulation with plasma from preeclamptic and eclamptic women, with the increment of NE concentration being greater with 50% than with 10% plasma. Moreover, 1% plasma from eclamptic and preeclamptic women and various concentrations of plasma from normotensive pregnant patients could not induce NE secretion. This implies that the increased concentration of NE in the preeclamptic and eclamptic condition is closely related to the severity of the disease. The failure of plasma from hypertensive pregnant women without superimposed preeclampsia demonstrates that effects of plasma from preeclamptic and eclamptic women relate to the disease and not just to the hypertension. Recently, we reported that plasma catecholamine levels are closely correlated to the degree of eclampsia. This result seems to be compatible with the findings of our present experiment.

Stimulation of the sympathetic nerve with a mixture of bupivacaine and eclamptic or preeclamptic plasma blunted the increased NE levels that have been seen in stimulation with plasma from eclamptic or preeclamptic patients only. Bupivacaine is thought to hamper the effect of plasma from preeclamptic and eclamptic women through the blockade of sympathetic stimulation. We also observed that incubation of cultured sympathetic neurons with bupivacaine significantly decreased NE secretion. The catecholamine transport system across the axoplasmic membrane is Na+-dependent and is blocked selectively by a number of drugs, such as bupivacaine. Local anesthetics block the conduction of nerve impulses by decreasing the permeability of excitable membranes to Na+, which produces a slight depolarization of the membrane. The blockade of depolarization affects the normal development of tyrosine hydroxylase activity and thus hampers NE secretion. Electron microscopic studies of bupivacaine-treated nerve cells showed demyelination of the nerve cells, and the myelin sheath appeared to be more sensitive. The demyelination of nerve cells implies the blockade of depolarization effects. Additional support for this hypothesis is derived from reports that lumbar epidural anesthesia blocks sympathetic nerve activities and thus improves the symptoms and biochemical parameters in preeclampsia. The results of the present study are compatible with those of previous reports, since our data show that stimulation with bupivacaine impaired NE secretion more than stimulation with eclamptic plasma, suggesting that hyperstimulation of the sympathetic nervous system in the eclamptic condition improves with bupivacaine treatment.

In conclusion, plasma from eclamptic and preeclamptic women has a potent depolarizing effect on the sympathetic nervous. Depression of the sympathetic nervous system may be an important treatment of preeclampsia.

Acknowledgments

This study was supported by a grant from the Japanese Ministry of Education and Science (No. 08457439) and the Adeza Biomedical grant (USA). We are indebted to Dr Sultana Jahan, Department of Obstetrics and Gynecology, and Dr Faruque A. Azim, Department of Pathology, Dhaka Medical College and Hospital, Bangladesh, for their contribution to the collection of blood samples and clinical details in Bangladesh. Yoko Kumakiri, Central Laboratory of Electron Microscopy, Hamamatsu University School of Medicine, Japan, is sincerely thanked for her expert technical assistance in the electron microscopic investigations.

References


Eclamptic Plasma Stimulates Norepinephrine Release in Cultured Sympathetic Nerve
Selina Khatun, Naohiro Kanayama, Eiji Sato, Hossain M. Belayet, Takao Kobayashi and Toshihiko Terao

Hypertension. 1998;31:1343-1349
doi: 10.1161/01.HYP.31.6.1343

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/31/6/1343

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/