Neutrophil Intracellular pH and Na^+/H^+ Exchanger Activity in Pre-eclampsia

Virginia M. Lee, Aidan W.F. Halligan, and Leong L. Ng

Elevated Na^+/H^+ exchanger activity and intracellular acidosis have previously been demonstrated in white blood cells isolated from women who have suffered from a pre-eclamptic pregnancy. The mechanisms underlying this abnormality and the implications in pre-eclamptic pregnancies are, at present, unclear. In this study, we used neutrophils from third trimester pre-eclamptic patients and third trimester normotensive pregnant controls to determine Na^+/H^+ exchanger isoform-1 (NHE-1) activity and intracellular pH. This was performed using a well-validated technique involving flurometry and a pH sensitive dye, 2,7'Bis-(carboxyethyl) 5.6 carboxyfluorescein acetomethyl ester (BCECF-AM). Time course experiments were performed to assess the contribution of plasma factors to intracellular pH measurements. Plasma digoxin-like factor (DLF) was assessed in both patients and normotensive controls. Neutrophil intracellular pH was significantly lower in the pre-eclamptic patients (7.15 ± 0.050) compared with the normotensive pregnant controls (7.36 ± 0.027; *P*<.001). NHE-1 activity (in mmol/L/min) was significantly higher in the pre-eclamptics (32.4 ± 1.9) compared with the normotensive neutrophils (27.1 ± 1.6; *P* = .038). Times course experiments showed that mean pre-eclamptic intracellular pH increased from 7.11 ± 0.049 to 7.25 ± 0.043 after 2 hours of incubation. DLF, measured as amount of inorganic phosphate liberated from adenosine triphosphate (ATP), was significantly lower when plasma from the pre-eclamptic patients was incubated with the enzyme compared with plasma from the normotensive pregnant women (54.9 ± 2.6 nmol/mL plasma *v* 63.91 ± 1.7 nmol/mL plasma, n = 6, *P* = .018 unpaired Student's *t* test). The results suggest that elevated NHE-1 activity and intracellular acidosis are intermediate phenotypes in women who have pre-eclampsia. Intracellular pH may have been affected by plasma as shown in the time course experiments. DLF, an inhibitor of Na^+/K^+ATPase, may contribute to this intracellular acidosis in pre-eclamptic neutrophils.

IN THE LEUKOCYTE, higher cellular sodium levels have been demonstrated in pre-eclampsia compared with normal controls, which suggests the possibility of alterations in cellular sodium transport pathways.[1] Cellular cation handling, especially in the kidney, plays an important role in both volume homeostasis and control of blood pressure. Changes have been described in normotensive pregnancy in the Na^+/K^+adenosine triphosphatase (ATPase), Na^+/K^+, Na^+/Li^+, and Na^+/H^+ exchangers.[2-5] Sodium electrochemical gradients across cell membranes are maintained primarily by the Na^+/K^+ATPase. This pump maintains low intracellular sodium and high intracellular potassium concentrations, while in the kidney, the same enzyme system mediates tubular sodium reabsorption. In normotensive pregnancy, the function of this enzyme is increased and results in a lowered intracellular sodium concentration and aids in gradual renal sodium retention.[6,7] Pre-eclampsia is associated with the presence of a circulating inhibitor of this pump, consequently resulting in a reduction in pump function and an increase in intracellular sodium.[8,9]

Because Na^+/H^+ exchanger isoform-1 (NHE-1) is responsible for a major component of cellular Na^+ influx, the role of NHE-1 in contributing to the elevated intracellular Na^+ in pre-eclampsia needs to be investigated. A small study in normotensive pregnancies suggested that NHE-1 activity in erythrocytes is elevated especially in the first trimester.[2] Only one study exists that assesses the role of NHE-1in pre-eclampsia.[10] No differences were described. This study was complicated by the use of "non-proteinuric" pre-eclamptics as the study group and the use of platelets as models.[10] The present study aims to provide comprehensive evidence of the status of NHE-1 in pre-eclampsia using well defined third trimester pre-eclamptic women and representative nucleated cells. We have previously documented an increased NHE-1 activity in postpartum pre-eclamptic women and suggested that abnormalities in the postpartum probably stem from the pre-eclamptic pregnancy or even before, which would suggest an inherent basis to the transport abnormality.[11] The intracellular pH in postpartum pre-eclamptic cells was significantly lower than that in postpartum normotensive control cells, and this study aims to identify the presence of this abnormality in pre-eclampsia.[11] We postulated that this lower intracellular pH may be due to the presence and effect of a circulating factor present in the plasma. Lopatin et al[12] suggested the presence of a digoxin-like factor (DLF) in plasma of women with pre-eclampsia. DLF inhibits Na^+/K^+ATPase activity and could result in a build up of protons triggered by an initial build up of sodium that activates Ca^{2+}/H^+ exchange, thus reducing intracellular pH. The presence of DLF may not only contribute towards this acidosis, but may contribute to the increased sensitivity of the vascular bed to pressor hormones and the hypovolemic hypertensive state, as well as causing vasoconstriction and increased blood pressure.[13]

Time course experiments were also performed to assess the effect of humoral and environmental factors on intracellular pH measurements. The presence of DLF was assessed by collecting extracts from whole plasma and analyzing the degree of inhibition of Na^+/K^+ATPase.

MATERIALS AND METHODS

Materials

The tissue culture medium 199 (TC199), nigericin, monensin, fatty acid free bovine serum albumin (BSA), 4-(2-hydroxyethyl)-1-pipera-

From the Departments of Medicine and Therapeutics and Obstetrics and Gynaecology, Leicester Royal Infirmary, Leicester, UK.

Submitted April 15, 2002; accepted July 23, 2002.

Address reprint requests to Leong L. Ng, MD, Department of Medicine and Therapeutics, Clinical Sciences Building, Leicester Royal Infirmary, Leicester LE2 7LX, UK.

0026-0495/03/5201-0009$35.00/0
doi:10.1053/meta.2003.50008

Table 1. Clinical Data for the Third Trimester Pre-eclamptic and Normotensive Control Patients

	Clinical Details	
	Pre-eclamptic	Normotensive
Patients (n)	17	17
Age (yr)		
Mean	28 (± 1.02)	29 (± 1.2)
Range	22-40	23-41
Urine (protein)		
Mean 24 hour	2.013 (± 0.38)	NA
Range	0.3-4.5	
BP booking (mm Hg)		
Mean	115/70 (± 3.6)	112/65 (± 1.9)
Range	100/60 to 140/85	100/60 to 120/80
BP highest (mm Hg)		
Mean	160/106 (± 2.9)	112/66 (± 1.5)*
Range	140/94 to 186/131	100/60 to 120/70
Gestational age at sampling		
Mean	35 (± 1.0)	36 (± 1.9)
Range	27-40	31-41
Smoke		
Yes	2	5
No	15	12
Family history		
PET	0	0
Hypertension	0	0
Gestation at delivery (wk)		
Mean	34 (± 1.0)	39 (± 0.24)†
Range	26 to 40	38 to 41
Sex of newborn		
Female	7	9
Male	10	8
Weight of newborn		
Mean	6 lb 4 oz (± 0.64)	8 lb (± 0.29)‡
Range	1 lb 6 oz to 9 lb 8 oz	6 lb 3 oz to 10 lb 4 oz

NOTE. Values are means with range and SEM.

Abbreviation: NA, not applicable.

*$P = .002$, †$P = .001$, and ‡$P = .035$, normotensive *v* pre-eclamptics by Student's *t* test.

zine-etanesulphonic acid (Hepes), 2,7′Bis-(carboxyethyl) 5,6 carboxyfluorescein acetomethyl ester (BCECF-AM), dextran, percoll, colored density marker beads, adenosine 5′-triphosphatase from porcine cerebral cortex, adenosine 5′-triphosphate, ouabain, were all obtained from Sigma Chemicals (Poole, Dorset, UK). The TC199 was adjusted to pH 7.4 using NaOH and contained 15 mmol/L HEPES and 1 g/L BSA. Penicillin and streptomycin were from GIBCO BRL (Uxbridge, Middlesex, UK). C_{18} Sep Pak cartridges were from Peninsular Labs (San Carlos, CA). All other chemicals were of AnalaR grade and were from BDH (Poole, Dorset. UK).

Patients

Seventeen third trimester pre-eclamptic women and 17 third trimester normotensive pregnant women matched for age and gestation were evaluated. Pre-eclampsia was defined according to the internationally accepted criteria of Davey and MacGillivray, which in brief, is where the cardinal features of the disorder, hypertension and proteinuria, occur for the first time after 20 weeks gestation with a diastolic blood pressure of more than 90 mm Hg and a total protein excretion of more than 300mg/24 hours in a previously normotensive woman.[14] Power analysis suggested that we need 17 subjects to demonstrate a standardized difference of 1.2 between the pre-eclamptic patients and the normotensive controls, with a power of 90% at $P < .05$. Clinical data were collected at the time of sampling and is summarized in Table 1. None of the pre-eclamptic women had a family history of hypertension, as this may have influenced the results. For each subject with pre-eclampsia, we identified an age-matched control, with a completely normal pregnancy. All of the subjects had their blood pressure measured at the time of the visit. Control subjects were normotensive throughout gestation, and none had a family history of pre-eclampsia, hypertension, or diabetes mellitus. The Leicestershire Ethics Committee approved the study, and all women who participated did so voluntarily, having given their informed consent.

Isolation of Neutrophils From Whole Blood

A total of 20 mL blood was collected from the forearm of patients in syringes containing 1 mmol/L sterile sodium citrate as the anticoagulant. Leukocytes were isolated by dextran sedimentation according to the method of Baron and Ahmed[15] and the contaminating erythrocytes were lysed by hypo-osmotic shock. Neutrophils were separated from the other cells using a self-generated continuous density gradient of a colloidal silica solution, Percoll. The self-generated continuous gradient was obtained by centrifugation of 8 mL of a 30% percoll solution at 21,982 × *g* for 15 minutes at 4°C. The density gradient was checked by the use of colored density marker beads. Once the red blood cells had been lysed, the leukocytes were resuspended in 1 mL sterile TC199 and applied to the density gradient. The gradient was then centrifuged at 482 × *g* for 15 minutes at 28°C to separate the neutrophils from the

other white blood cells in the pellet. The neutrophils were then washed, and viability of the isolated cells was consistently more than 95%, as demonstrated by the dye exclusion test with trypan blue.

Measurements of Intracellular pH and Na^+/H^+ Exchanger Activity

Isolated leukocytes were loaded with 5 μmol/L of the pH sensitive fluorescent dye, BCECF-AM, in TC199 for 30 minutes at 37°C. The cells were then left for 15 minutes to allow for complete de-esterification of the fluorophore. Methods of intracellular pH and Na^+/H^+ exchanger activity have previously been described in detail.[11] Briefly, intracellular pH was measured in a buffer consisting of NaCl 140 mmol/L, KCl 5 mmol/L, $MgSO_4$ 0.8 mmol/L, $CaCl_2$ 1.8 mmol/L, glucose 5 mmol/L, and HEPES 15 mmol/L, pH 7.4. The measurements were performed in a 37°C thermostatically controlled sample compartment holder within a dual grating fluorometer (Deltascan: Photon Technology, South Brunswick, NJ) with dual wavelength excitation at 500 and 439 nm (slit width, 2.5 nm) and the emission wavelength set at 530 nm (slit width, 10 nm). Calibration employed a double ionophore technique (nigericin and monensin).[16]

NHE-1 activity was measured by clamping the cells at pH_i 6.0 because the exchanger operates near its maximal transport capacity when its pH is near 6.0.[17] The exchanger was activated in a buffer identical to that used for measuring the resting intracellular pH, but with the addition of the ionophores nigericin (2 μmol/L) and monensin (5 μmol/L), adjusted to a pH of 6.0 as described previously.[11]

To calculate NHE-1 activity, it was necessary to determine Na^+ independent H^+ efflux. N-methyl-D-glucamine chloride (NMG) (133 mmol/L final concentration, pH 7.4) was added as a substitute for Na^+ allowing the determination of the efflux of H^+ that is not due to the activity of NHE-1.[18] The buffering capacity was measured at pH 6.0 by recording the pH change induced by the addition of extracellular NH_4Cl (50 mmol/L final concentration).[16] These measurements were performed in triplicate to assure reproducibility. Time course experiments were performed after the initial assessment of the intracellular pH. Neutrophils were incubated at 37°C for 2 hours and then analyzed once more. This would allow the cells to be examined away from the influences of "plasma factors." The cells would have been isolated from the whole blood for a further 2 hours. Any differences may provide an indication to the humoral influences on the measurements.

Assessment of DLF in Plasma

Peripheral blood samples were drawn from patients into tubes containing 4 mmol/L EDTA, and the plasma was collected by centrifugation of the samples at 1,709 $\times$ *g* for 20 minutes. Disposable C_{18} SepPak cartridges were preconditioned with 4 mL 25% acetonitrile and 20 mL 0.1% trifluoroacetic acid (TFA). Plasma was passed through the column, and the column was then washed thoroughly with 0.1% TFA,and eluted with 3 mL 25% acetonitrile. Recovered eluate was evaporated to dryness under vacuum and stored at −80°C. The extracts were reconstituted in ATPase buffer (NaCl 100 mmol/L, KCl 10 mmol/L, $MgCl_2$ 4 mmol/L, EGTA 0.2 mmol/L, Tris/Base 30 mmol/L, Histidine/Base 5 mmol/L, pH 7.8 HCl). The ionic composition of this buffer was largely based on the optimal conditions for measurement of leukocyte Na^+K^+ATPase activity reported by Baron and Khan.[19] A total of 100 μL reconstituted plasma was incubated with 400 μL ATPase solution containing 2 mU Na^+/K^+ATPase (pig cerebellum) and left for 20 minutes at 37°C. A total of 100 μL ATP (buffered to pH 7.8 with Tris/Base) was added, making a final volume of 0.6 mL and a final ATP concentration of 4 mmol/L. The tubes were incubated in the waterbath at 37°C with gentle agitation for 30 minutes. The reaction was stopped by adding 0.4 mL sodium dodecyl sulfate (SDS) 100 g/L. This stops the enzyme activity very effectively as the inorganic phosphate does not increase by more than 1% over the subsequent hour. To measure inorganic phosphate in supernatants, a more sensitive version of the Fiske-Subbarow method was adopted. Stannous chloride was made as follows. A total of 28 mL concentrated sulphuric acid was added to 700 mL distilled water and left to cool at room temperature. Two grams hydrazine sulfate were added, and the volume made up to 1 L with distilled water. A total of 200 μg stannous chloride was added to the liter, and the solution was filtered. The acid molydbate solution was made by adding 35 mL concentrated sulphuric acid to 700 mL distilled water. After cooling, 10 g of ammonium molydbate was added, and the solution made up to 1 L with distilled water. Into the tubes containing the ATPase buffer, plasma and SDS (total, 1 mL). One milliliter stannous chloride was added, and the tubes were then mixed. One milliliter acid molydbate solution was added. The tubes were left for 15 minutes, and the OD_{640nm} was read on a spectrophotometer. Sample determination was performed by subtracting the OD_{640nm} of the blank control from the OD_{640nm} of the test samples. A standard curve was constructed with dilutions of a 1 mol/L sodium dihydrogen orthophosphate solution. Inorganic phosphate concentration was then read from the standard curve, and the results were expressed as nanomoles inorganic phosphate/mL plasma. This represented the degree of inhibition by factors present in the reconstituted plasma because an inhibited Na^+/K^+ATPase would result in less inorganic phosphate being liberated according to the following reaction:

$$ATP + H_2O \xrightarrow{\text{ATPase}} ADP + Pi$$

Statistics

Results are expressed as means ± SEM. Normality of data was established and comparisons by unpaired and paired Student's *t* test or analysis of variance (ANOVA) of means was performed using an OXSTAT statistical package (Microsoft, Reading, Berks, UK). Two-tailed probability values less than .05 were considered significant.

RESULTS

Intracellular pH

The results for intracellular pH are shown in Fig 1. The mean intracellular pH of the 2 groups was significantly different ($P <$ .001). The mean value for the normal control neutrophils was 7.36 ± 0.027. Patients with pre-eclampsia had neutrophils, which were significantly more acidotic, with a mean intracellular pH of 7.15 ± 0.050.

Buffering Capacity

The results for buffering capacity are shown in Fig 2. The buffering capacity of neutrophils from pre-eclamptic patients (40 ± 3.90 mmol/Ll.pH unit) were significantly elevated compared with the normotensive patients (26 ±1.3 mmol/Ll.pH unit P = .005).

Na^+/H^+ Exchanger Activity

Figure 3 demonstrates the neutrophil NHE-1 activity after clamping intracellular pH to 6.0, near the V_{max}. The mean NHE-1 activity was significantly higher in the pre-eclamptic neutrophils (32.4 ± 1.9 mmol/L · min) compared with the control neutrophils (27.1 ± 1.6 mmol/L · min, P = .038).

Effect of Humoral Influences on Intracellular pH

Figure 4 shows the effect of plasma factors on intracellular pH. Normal pregnant and pre-eclamptic intracellular pH were

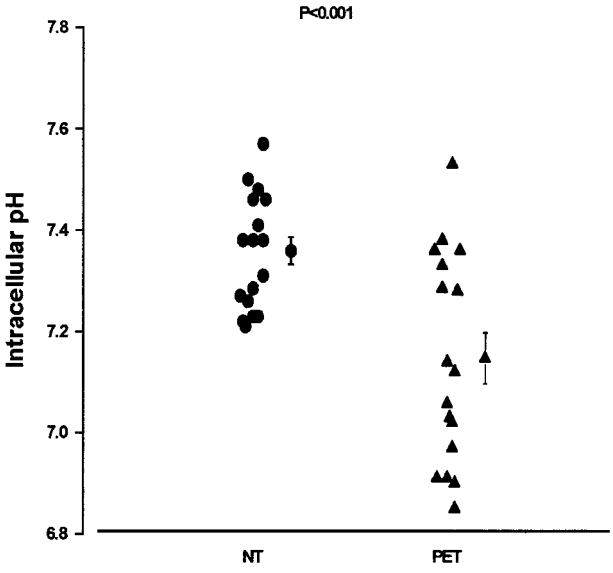

Fig 1. Plot of neutrophil intracellular pH in third trimester normotensive (NT) and pre-eclamptic (PET) patients. The mean and SEM are plotted. PET patients exhibit a lower intracellular pH (7.15 ± 0.050) compared with NT patients (7.36 ± 0.027, $P < .001$, Student's *t* test).

measured immediately after neutrophil isolation and 2 hours later. Mean pregnant control pH showed no significant differences, whereas mean pre-eclamptic intracellular pH increased from 7.11 ± 0.049 to 7.25 ± 0.043, $P = .01$. Pre-eclamptic women showed a significant increase in intracellular pH when measured 2 hours after cellular isolation.

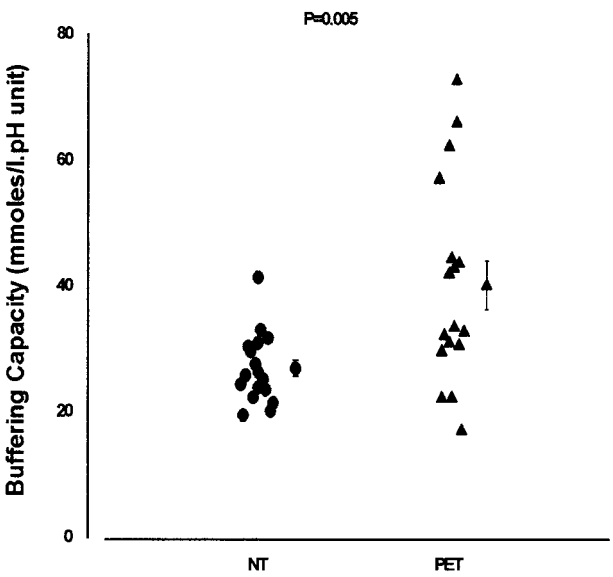

Fig 2. Plot of neutrophil buffering capacity measured at pH_i 6.0 in third trimester NT and PET patients. The mean and SEM are plotted. The buffering capacity of leukocytes from PET patients (40 ± 3.9) was significantly elevated compared with NT patients (26.82 ± 1.3, $P = .005$, Student's *t* test).

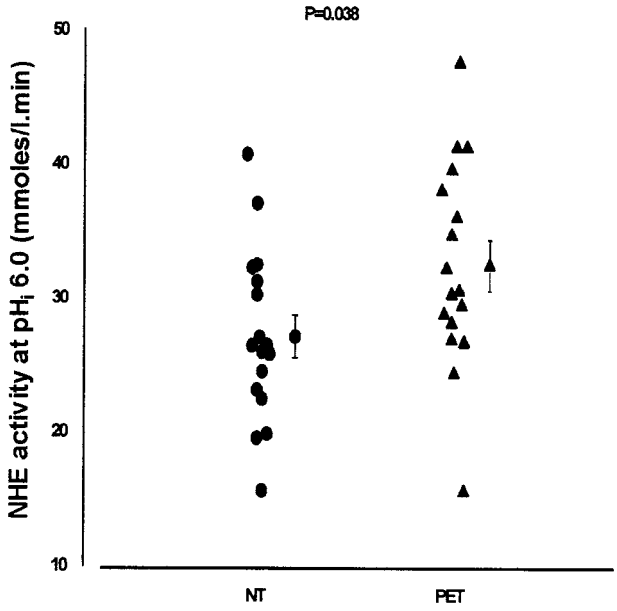

Fig 3. Plot of neutrophil Na^+/H^+ exchanger activity in third trimester NT and PET patients. Na^+/H^+ exchanger activity was determined after clamping pH_i to 6.0, near the V_{max} of the exchanger of leukocytes. The mean and SEM are plotted. The Na^+/H^+ exchanger activity of neutrophils from PET patients (32.35 ± 1.9) was significantly elevated compared with NT patients (27.09 ± 1.6, $P = .038$, Student's *t* test).

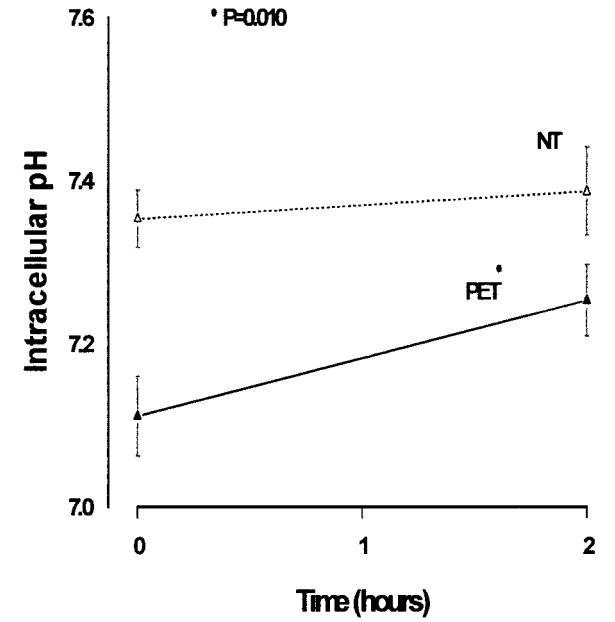

Fig 4. Neutrophil mean pH_i of both third trimester NT and PET patients is plotted against time after cell isolation. There is a significant increase in pH_i from 0 to 2 hours in the PET patients (7.11 ± 0.0490 *v* 7.25 ± 0.0438, $P = .010$, paired Student's *t* test).

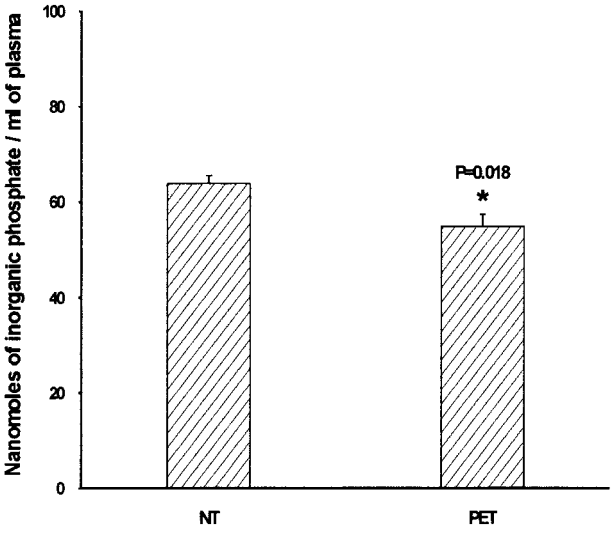

Fig 5. DLF activity presented as the amount of inorganic phosphate liberated when plasma was incubated with the Na^+/K^+ATPase isolated from pig cerebellum. Plasma from NT and PET pregnant women was analyzed, and there was a significant decrease in the inorganic phosphate measured when pre-eclamptic plasma was incubated with the enzyme compared with plasma from NT pregnant women (54.9 ± 2.6 nmol/mL plasma *v* 63.91 ± 1.7 nmol/mL plasma, n = 6, *P* < .018 unpaired Student's *t* test).

Measurement of DLF

Figure 5 shows the ability of extracted plasma from both third trimester pre-eclamptic and third trimester normal pregnant women to inhibit the Na^+/K^+ATPase-mediated hydrolysis of ATP. The presence of a DLF was reflected by the amount of inorganic phosphate liberated from ATP with less phosphate representing inhibition of the enzyme and hence more DLF. Inorganic phosphate was significantly lower when plasma extracts from the pre-eclamptic women was incubated with the enzyme compared with plasma extracts from the normotensive pregnant women (54.9 ± 2.6 nmol/mL plasma *v* 63.9 ± 1.7 nmol/mL plasma, $P = .018$ unpaired Student's *t* test).

DISCUSSION

Alterations in NHE-1 activity have consistently been reported in essential hypertension in a variety of cells types.[20-22] In this study, we have examined the activity of NHE-1 in third trimester pre-eclamptic and normotensive pregnant control women. The data presented provide evidence that NHE-1 activity is elevated in pre-eclampsia with respect to normotensive control pregnancy.

It is the first time that this membrane abnormality has been demonstrated in women with pre-eclampsia. Only one study to date exists concerning the activity of the exchanger in pre-eclampsia, and no differences were identified between the pre-eclamptic and control groups.[13] These finding are in disagreement with the findings of our study. The reasons for the differences are perhaps due to the inclusion criteria of the patient group. The study by Graham et al[10] considered whether NHE-1 activity was affected in non-proteinuric pre-eclampsia. We used tightly defined patients according to the criteria for diagnosing pre-eclampsia. Second, we used nucleated cells rather than platelets and a well-established technique to assess the status of NHE-1 activity. The cause of the enhanced NHE-1 activity in cells from pre-eclamptic patients is unknown, but possible mechanisms concerning the enhanced activity observed in the postpartum pre-eclamptic group were discussed previously.[11] Phosphorylation of NHE-1 has previously been shown to be increased in essential hypertension.[20] It is possible that alterations in phosphorylation could contribute to the increased NHE-1 activity demonstrated in pre-eclamptic cells. This hypothesis can be tested by immunoprecipitation of NHE-1 protein from ^{32}P-labeled cells and remains to be examined further in neutrophils from pre-eclamptic and normotensive controls. It has been demonstrated that the phosphorylation of serine residues increases parallel to exchanger activation making it a likely mechanism for increased activity.[23] It seems that the same phosphorylation pattern is implicated regardless of the signalling pathway stimulated, which has led to the proposed existence of a NHE-1 kinase, in which all pathways converge culminating in the phosphorylation of NHE-1 protein.[24-26] An increase in NHE-1 activity could result in a build up of Na^+, particularly because pre-eclampsia is associated with reduced Na^+K^+ATPase activity.[4] This Na^+ overload may result in increased intracellular Ca^{2+} as a consequence of alterations in Na^+/Ca^{2+} exchange. This may result in an increase in vascular tone and peripheral resistance possibly contributing to the hypertension associated with pre-eclampsia.

The buffering capacity is increased in pre-eclampsia along with a lower intracellular pH, findings consistent with the results presented previously by our group.[11] The increased cellular buffering capacity could be a response to counteract any upregulation in intracellular acid production in these cells. It is unclear which mechanisms are responsible for the intracellular acidosis. Is it a part of the pre-eclamptic phenotype or induced by the pregnancy itself? Experiments were performed to assess the effect of the plasma on the measurements of intracellular pH. Cells were isolated and separated from whole blood. Measurements were performed immediately and after 2 hours. This small study provided some interesting results concerning the nature of this intracellular acidosis reported in pre-eclampsia. The results suggest a factor(s) in the pre-eclamptic plasma that influences the intracellular pH. Intracellular pH starts to approach values near that obtained for normotensive controls when removed from the plasma for 2 hours. Pre-eclampsia has previously been associated with the presence of an endogenous DLF that circulates in the plasma.[12] Extracts from pre-eclamptic plasma inhibited Na^+/K^+ATPase. Our study suggests that DLF, characterized by its ability to inhibit Na^+/K^+ATPase, is present in normal plasma, but is significantly increased in pre-eclamptic plasma. These findings complement the work of others.[12-13] It is possible that the presence of this factor in the pre-eclamptic plasma may be associated with the lower intracellular pH.

There are 2 potential explanations for the intracellular acidosis in pre-eclampsia. First, it may be secondary to the changes in other intracellular ions, such as Na^+ and Ca^{2+}. Evidence in support of this argument comes from the work of Souza et al,[27] who examined the involvement of intracellular pH and Ca^{2+} in Na^+/K^+ATPase inhibition in cardiac myo-

cytes. They reported that the addition of 100 μmol/L ouabain (an inhibitor of the Na^+/K^+ATPase) to myocytes resulted in a large increase in $[Ca^{2+}]_i$ (200%) and a decrease in pH_i. The intracellular acidification was prevented by the removal of extracellular calcium, and inhibition of the Na^+/H^+ exchanger had no effect on the ouabain-induced acidification. This suggested that the intracellular acidification was mediated by an initial Ca^{2+} influx via Na^+/Ca^{2+} exchanger that may activate the Ca^{2+}/H^+ exchange system.[27] These data suggest that the presence of the digitalis-like factor in pre-eclamptic plasma may be partially responsible for the acidic pH that is a characteristic of the neutrophils isolated from these pre-eclamptic patients. In addition, there may be a primary abnormality involving intracellular Ca^{2+} regulation, because several studies have suggested that intracellular Ca^{2+} is increased in pre-eclamptic cells.[28,29] Perhaps this abnormality is fundamental to the acidosis, because an increase in intracellular Ca^{2+} may directly activate the Ca^{2+}/H^+ exchanger and thereby increase the intracellular proton content and thus lower the intracellular pH. There is little evidence to suggest increased Ca^{2+}/H^+ exchange activity and, therefore, this may be an area for further investigation. Second, if the primary abnormality is indeed intracellular acidosis, then neutrophil Ca^{2+}/H^+ may be activated and subsequently lead to an influx of calcium, which would activate calcium-dependent cell signals, such as protein kinase C and the production of vasoactive substances (nitric oxide plasminogen activator inhibitor-1, and endothelin) that may be important in the pathophysiology of pre-eclampsia, because it has been agreed that the synthesis and release of certain vasoactive compounds can be modulated by changes in intracellular calcium.[30] In both possibilities, it is likely that the intracellular acidosis is causal or a result of increased intracellular Ca^{2+}, and it is this latter cation that may be involved in alterations of vascular contractility.

In conclusion, pre-eclamptic patients have a lower intracellular pH compared with normotensive pregnant controls. Intracellular pH was affected by a plasma factor(s) and DLF, an inhibitor of Na^+/K^+ATPase, may be implicated. The NHE-1 activity and buffering capacity were increased in pre-eclamptic neutrophils compared with neutrophils from control women. The importance of the NHE-1 activity in the pathogenesis of pre-eclampsia remains enigmatic. This newly recognized intermediate phenotype of enhanced NHE-1 activity and lowered intracellular pH may be a marker for the disease, but its mechanism, particularly with regard to intracellular Ca^{2+} regulation, remains to be fully elucidated.

REFERENCES

1. Seon R, Forrester T: Relationship between leucocye sodium content and high blood pressure during development and resolution of pre-eclampsia. Clin Sci 76:199-203, 1989

2. Matteucci E, Giampietro O: Activity of erythrocyte sodium hydrogen exchange in normal pregnancy. Clin Sci 93:431-434, 1997

3. Worley RJ, Hentschel WM, Cormier C, et al: Increased sodium-lithium countertransport in red cells of patients with essential hypertension. N Engl J Med 302:772-776, 1980

4. Heilmann L, VonTempelhoff GF, Ulrich S: The Na^+/K^+ cotransport system in erythrocytes from pregnant patients. Arch Gynecol Obstet 253:167-174, 1993

5. Lindheimer MD, Katz AI: Kidney function in the pregnant rat. J Lab Clin Med 78:633-641, 1971

6. Gallery EDM, Rowe J, Brown MA, et al: Effects of changes in dietary sodium on active electrolyte transport by erythrocytes at different stages of human pregnancy. Clin Sci 74:145-150, 1988

7. Tranquilli AL, Mazzanti L, Bertoli E, et al: Sodium/potassium-adenosine triphosphatase on erythrocyte ghosts from pregnant women and its relationship to pregnancy induced hypertension. Obstet Gynecol 71:627-630, 1988

8. Kaminski K, Rechberger T: Concentration of digoxin-like immunoreactive substance in patients with pre-eclampsia and its relation to severity of pregnancy-induced hypertension. Am J Obstet Gynecol 165:733-736, 1991

9. Miyamoto S, Makino N, Shimokawa H, et al: The characteristics of erythrocyte Na^+ transport systems in normal pregnancy and pregnancy induced hypertension. J Hypertens 10:367-72, 1992

10. Graham D, Kingdam JC, McDonald J, et al: Platelet sodium/hydrogen ion exchange in normal pregnancy and non proteinuric pre-eclampsia. J Hum Hypertens 11:453-456, 1997

11. Lee VM, Halligan AW, Ng LL: Leucocyte intracellular pH and Na+/H+ exchanger isoform-1 activity in postpartum women with pre-eclampsia. Br J Obstet Gynaecol 108:615-22, 2001

12. Lopatin DA, Ailamaziam EK, Dmitrieva RI, et al: Circulating butodiendide and cardenolide sodium pump inhibitor in pre-eclampsia. J Hypertens 8:1179-1187, 1999

13. Gregoire I, Roth D, Siegenthaler G, et al: A Ouabain-displacing factor in normal pregnancy, pregnancy induced hypertension and pre-eclampsia. Clin Sci 74:307-310, 1988

14. Davey DA, MacGillivray I: The classification and definition of the hypertensive disorders of pregnancy. Am J Obstet Gynecol 158: 892-898, 1988

15. Baron DN, Ahmed SA: Intracellular concentrations of water and of the principal electrolytes determined by analysis of isolated human leucocytes. Clin Sci 37:205-219, 1969

16. Ng LL, Dudley C: Intracellular pH clamping of human leucocytes: A technique for determination of cellular buffering power and Na^+ H^+ antiport characteristics. Clin Sci 77: 417-423, 1989

17. Ng LL, Bomford J: Altered stoichiometry of the human leucocyte Na^+/H^+ antiport with decreasing pH. J Biochemistry 259:311-314, 1989

18. Reuss F, Finn AL: Electrical properties of the cellular transepithelial pathway in Necturus gall bladder. I .Circuit analysis and steady state effects of mucosal solution ionic substitutions. J Membr Biol 25:115-139, 1975

19. Baron DN, Khan FA: Optimal conditions for measurement of Na+,K+-ATPase activity of human leucocytes. Clin Sci 68:143-149, 1985

20. Ng LL, Sweeney FP, Siczkowski M, et al: Na^+/H^+ antiporter phenotype, abundance, and phosphorylation of immortalized lymphoblasts from humans with hypertension. Hypertension 25:971-977, 1995

21. Ng LL, Simmons D, Frighi V, et al: Effect of protein kinase C modulators on the leucocyte Na^+/H^+ antiport in type I (insulin-dependent) diabetic subjects with albuminuria. Diabetologia 33:278-284, 1990

22. Rosskopf D, Dusing R, Siffert W: Membrane sodium proton exchange and primary hypertension. Hypertension 21:607-617, 1993

23. Sardet C, Fafournoux P, Pouyssegur J: Alpha-thrombin, epidermal growth factor, and okadaic acid activate the Na+/H+ exchanger, NHE-1, by phosphorylating a set of common sites. J Biol Chem 266:19166-19171, 1991

24. Sardet C, Counillion L, Franchi A, et al: Growth factors induce

phosphorylation of the Na^+/H^+ antiporter, a glycoprotein of a 100 kDa. Science 247:723-726, 1990

25. Bianchini L, Woodside M, Sardet C, et al: Okadaic acid, a phosphatase inhibitor induces activation and phosphorylation of the Na^+/H^+ antiport. J Biol Chem 266:15406-15413, 1991

26. Wakabayashi S, Sardet C, Fafournoux P, et al: The Na^+/H^+ antiporter domain mediates growth factor signals and controls H^+-sensing. Proc Natl Acad Sci USA 89:2424-2428, 1992

27. Souza MM, Gross S, Boyle RT, et al: Na^+/K^+ATPase inhibition during cardiac myocyte swelling: Involvement of intracellular pH and Ca^{2+}. Mol Cell Biochem 210:173-183, 2000

28. Ray J, Vasishta K, Kaur S, et al: Calcium metabolism in pre-eclampsia. Int J Gynaecol Obstet 66:245-250, 1999

29. Hojo M, Suthanthiran M, Helseth G, et al: Lymphocyte intracellular free calcium concentration is increased in preeclampsia. Am J Obstet Gynecol 180:1209-1214, 1999

30. Nilius B, Droogmans G: Ion channels and their functional role in vascular endothelium. Physiol Rev 81:1415-59, 2001