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Na^+-H^+ exchange activity throughout pregnancy: the proper experimental approach

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Summary. - Pregnancy is associated with a 30-50% rise in cardiac output and a close to 50% increase in blood volume. The Na^+-H^+ exchanger is a key mediator of tubular NaCl absorption and a stimulus-response coupling mediator. We measured erythrocyte Na^+-H^+ exchange activity over the course of normal pregnancy in 18 healthy pregnant women (mean age 32 ± 4 years) at 14, 24 and 33 weeks of gestation and 15 nonpregnant healthy women (mean age 32 ± 9 years). No pregnancy was complicated by hypertension. Serum urea, creatinine and sodium did not change through gestation, while serum potassium slightly but significantly decreased. Urinary excretion rates of both sodium and potassium remained unchanged. Urea and creatinine clearances were constantly elevated in pregnant *vs* non-pregnant control women. Erythrocyte Na^+-H^+ exchanger reached the highest activity at about the 14th week of gestation, when cardiac output also peaked. Thereafter, it tended to decrease, yet remaining above the normal values until the 34th week. Conceivably, the observed hyperactivity of the transporter may be a contributing factor to the hemodynamic adjustments attending to normal pregnancy.

Key words: pregnancy, sodium-hydrogen antiporter.

Riassunto *(L'attività dell'antiporto Na^+-H^+ in corso di gravidanza: il migliore approccio sperimentale).* - La gravidanza si accompagna ad un aumento della gittata cardiaca del 30-50% e del volume ematico pari a circa il 50%. L'antiporto Na^+-H^+ ha un ruolo chiave nel riassorbimento tubulare del NaCl e nella transduzione dei segnali a livello cellulare. Abbiamo studiato l'andamento dell'antiporto Na^+-H^+ eritrocitario nella gravidanza in 18 gravide sane (età media 32 ± 4 anni) a 14, 24 e 33 settimane di gestazione e 15 donne sane non gravide (32 ± 9 anni). Nessuna gravidanza risultava complicata da ipertensione. Le concentrazioni sieriche di urea, creatinina e sodio non variavano sensibilmente durante la gestazione, mentre la potassiemia diminuiva significativamente. L'escrezione urinaria di sodio e potassio rimaneva invariata. Le clearances di urea e creatinina risultavano costantemente elevate nelle gravide rispetto ai controlli. L'attività dell'antiporto raggiungeva i valori più alti a circa 14 settimane di gravidanza, quando anche la gittata cardiaca presentava il proprio picco massimo. Successivamente si riduceva, pur sempre rimanendo superiore alla norma fino alla 34ª settimana. Tale iperattività del trasportatore ionico può naturalmente contribuire agli adattamenti emodinamici che si verificano nel corso della gravidanza.

Parole chiave: gravidanza, antiporto sodio-idrogeno.

Introduction

Pregnancy causes a number of physiologic changes in all organ systems. From the cardiovascular viewpoint, a 30 to 50% rise in cardiac output occurs, beginning by 6 week of pregnancy and peaking between 12 and 20 week. Thereafter, cardiac output remains elevated until after 30 week, when it may decrease slightly due to vena cava obstruction by the enlarging uterus. Blood volume increases proportionally with cardiac output, but the increase in plasma volume is greater, close to 50%, than the increase in red blood cell (RBC) mass, about 25% [1]. Potential pathways that might account for the observed plasma volume expansion remain to be identified. Glomerular filtration rate increases 30 to 50%, peaking between 16 and 24 week of pregnancy; renal plasma flow rises correspondingly. Mechanical and hormonal influences dilate the excretory tract. These increases in kidney function cause blood urea and creatinine levels to drop [2]. The sodium-hydrogen exchanger or antiport is a secondary active transporter driven by the primary Na-K-ATPase [3]. It is involved in proton transport [4] and directly controls such as intracellular pH, transmembrane pH and electrical gradients. In non-epithelial cells, the antiport is quiescent at the physiological pH_i; it becomes activated when the cytosol has been acidified [5]. Renal brush-border sodium-hydrogen exchanger has known transport properties: it may transport multiple pairs of cations in addition to Na^+ and H^+, such as Li^+ and $NH4^+$; amiloride acts as an external competitive inhibitor of the exchanger; internal H^+ interacts at a modifier site finally activating the transport system; the H^+ affinity of the modifier site may be altered by many regulators such as growth factors

and angiotensin [5]. Sodium-hydrogen exchanger could be a key mediator of NaCl absorption in the proximal tubule: the transporter is regulated both by acid-base perturbations and by volume-regulatory hormones and agonists. Four (possibly five) isoforms have been identified, different as for amino acid composition, functional properties and tissue distribution [6]. NHE1 is ubiquituosly expressed: in the kidney, the protein resulted more abundant in basolateral membrane-enriched fraction isolated from the renal cortex [7]. NHE1 is also present in erythrocytes where it accounts for amiloride-sensitive Na^+-H^+ activity [8]. The relationship between sodium-hydrogen exchange and RBC sodium-lithium countertransport has never been clarified. It has been proposed that the overactive sodium-lithium countertransport in essential hypertension could reflect a mode of operation of plasma membrane sodium-hydrogen exchangers, whose activity has been confirmed elevated in that pathology by numerous studies. Data from Rutherford *et al.* [8] did not support this hypothesis: NHE3, the less sensitive isoform to amiloride inhibition, was undetectable in the erythrocytes, thus making unlikely the identity of the amiloride-insensitive sodium-lithium countertransport with the amiloride-sensitive sodium-hydrogen antiport. Previous investigations concerning monovalent cation fluxes in pregnancy examined only the activity of sodium-lithium countertransport in erythrocytes of normal and hypertensive pregnant women [9-15]. Countertransport activity resulted elevated during normal pregnancy, the major increase occurring in the first trimester. Most studies did not find any detectable difference between normotensive and hypertensive pregnant women. Factors that account for the increase in countertransport activity with pregnancy remain unknown: the concentration of many hormones is rising in maternal plasma as well as the plasma lipid profile changes markedly during this time. Prompted by these findings, we aimed to explore the contribution of adaptations in sodium-hydrogen exchanger activity to physiologic cardiovascular changes during pregnancy. The complexity of the picture associated with pregnancy needed a wide-ranging protocol to succeed in analyzing as many metabolic features as possible repeatedly throughout gestation. Plasma lipids had to be monitored: cell membrane lipid components are in dynamic equilibrium with plasma lipids. Hence, plasma lipids may influence the cell membrane composition and functional properties of its cation transport systems. However, they cannot be the main determinants of these changes in transporter activity, but only contribute to the environmental aspect of membrane ion transport variability [16]. In the present work, our aim was to achieve an experimental approach to monitor, throughout gestation, the sodium-hydrogen antiport activity on isolated erythrocytes from pregnant women who were at the same time studied as for renal functional adaptations (clearances and handling of electrolytes) as well as for glucose tolerance and plasma lipid profile changes.

Methods

Erythrocyte sodium-hydrogen exchanger

The expression of the transporter in the circulating erythrocyte makes disposable an accessible cell for clinical studies. The different analytical approaches and the coexistence of multiple proton translocating systems across red cell membrane make problematic a quantitative measurement of the rates of ion fluxes [17, 18]. Beyond the intrinsic technical problems, we did need an analytical procedure suitable for cross-sectional as well as longitudinal clinical studies: it would be simple, rapid, reproducible, inexpensive. We measured sodium-hydrogen exchange rate according to Orlov *et al.* [19]: this procedure had previously proved in our hands to be provided with the required properties [20]. Following platelet removal, erythrocytes were isolated by three successive washes with cold isotonic saline solution (5 mmol/l sodium phosphate, pH 7.4, 1:4 cell to buffer ratio). Packed cells (0.2 ml) were added to 3.8 ml of a solution containing 150 mmol/l NaCl, 1 mmol/l KCl, 1 mmol/l $MgCl_2$, 10 mmol/l glucose, and were incubated at 37 °C for 5 min under magnetic stirring. Basal erythrocyte number and volume had been determined to correct flux results by the real hematocrit. The measured hematocrit, corrected by dilution, resulted in 81.9±10.8% (4.1 ± 0.5% final packed cell volume in the incubation mixture). The pH of the cell suspension was adjusted to 6.35-6.45 within about 10 min by 0.2 mol/l HCl solution. Successively, we added DIDS (0.2 mmol/l final) and adjusted the pH of the medium to 7.95-8.00, as soon as possible, by 0.05 mol/l NaOH solution. In a parallel experiment, amiloride (0.5 mmol/l) was added with 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid (DIDS). Thereafter, proton efflux was registered. The rate of Na^+-H^+ exchange, mmol/l of cells per h, derives from the difference of rates of medium acidification with and without amiloride, corrected by the buffer capacity of the incubation medium (b), the cell volume in the suspension (m) and the incubation time (t), according to the formula $V=(\Delta pH_1-\Delta pH_2)\ b\ m^{-1}\ t^{-1}$. ΔpH_1 and ΔpH_2 were calculated both as the initial (1st min) rates of the medium acidification in the absence and presence of amiloride, respectively. Buffer capacity was determined by titration of the incubation medium with NaOH and HCl solution in the explored pH range. Parameter b ranged between 0.211-0.421 (0.261 ± 0.046) μEq hydrogen ions per pH unit.

Routine assays

Serum and urine urea, creatinine, glucose, cholesterol, triglycerides, electrolytes were measured by BM/ Hithachi System 717 model and reagents from Boehringer Mannheim GmbH (Mannheim, Germany). HDL cholesterol was measured after precipitation with phosphotungstic acid and using reagents from Menarini SpA (Milan, Italy). HbA1c was evaluated by Bio-Rad DIAMAT ™ fully automated glycosylated hemoglobin Analyzer System. Serum insulin level was measured by radioimmunoassay.

Study group

Initially, we intend to study normotensive nondiabetic pregnant women and healthy nonpregnant controls. Successively, only when we really know the physiologic behaviour of the pump in normal condition as well in normal pregnancy, we will extend the study to hypertensive and diabetic pregnancies.

Protocol: prospective assessment

Pregnant women have to be studied repeatedly during gestation, at the first, second, third trimesters. At each antenatal visit, body weight and blood pressure are recorded. Blood and 24 h urine samples are taken to perform laboratory measurements: clearances of urea and creatinine; serum levels and urinary excretion rates of sodium and potassium; hemoglobin A1c. Maternal glucose tolerance is evaluated by 100 g 3 h oral glucose tolerance test: areas under both glycemic and insulinemic curves are geometrically calculated. At the 2nd and 3rd visit, mothers undergo also a real-time ultrasonic examination: the biparietal diameter (BPD) of the fetal head, abdominal circumference (AC) and femur length (FL) are recorded. At delivery, newborn clinical data are registered.

Preliminary results

Preliminary results from 18 young pregnant women and 15 matched healthy control women confirm previous data as for renal functional adaptation to pregnancy and evidence original findings as for sodium-hydrogen activity. Serum urea, creatinine and sodium did not change through gestation, while serum potassium slightly but significantly decreased from the first to the third trimester. Urinary excretion rates of both sodium and potassium remained unchanged. Urea and creatinine clearances were constantly elevated in pregnant versus nonpregnant control women. Erythrocyte Na^+-H^+ exchange activity resulted significantly higher at each trimester than in control subjects. Thus, the exchanger reached the highest activity at about the 14th week of gestation, when cardiac output also peaked. Thereafter, it tended to decrease, yet remaining above the normal values until the 34th week. Conceivably, the observed hyperactivity of the transporter may be a contributing factor to the hemodynamic adjustments attending to normal pregnancy.

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