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Sodium regulation, sodium pump function and sodium pump inhibitors in uncomplicated pregnancy and preeclampsia

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1. ABSTRACT

Preeclampsia is a disease characterized by hypertension and proteinuria but can manifest many abnormalities. Some of the best documented alterations involve changes in the handling of sodium ion both on the systemic and on the cellular level. There is broad agreement that the components of the renin-angiotensin-aldosterone pathway are markedly reduced in women with preeclampsia. However, other changes, especially those involving cell sodium are less consistent. A majority of studies support an increase in peripheral cell sodium concentration. This would suggest a defect in (Na,K)ATPase or sodium pump activity. Direct study of cellular sodium pump activity provides suggestive but not unequivocal support for this decreased sodium pump activity. Other evidence indicates increased circulating concentrations of a sodium pump inhibitor in most, but not all, studies of preeclampsia. Together, current research argues more strongly in favor of derangements of cell sodium handling perhaps mediated by circulating sodium pump inhibitors leading often to increased cell sodium. Such an increase of cell sodium in vascular tissue has previously been shown to enhance vascular sensitivity to vasoconstrictor agents or lead directly to increased vasoconstriction.

2. INTRODUCTION

Preeclampsia (PE) differs from uncomplicated pregnancy in a remarkable number of ways. The sheer number of abnormalities associated with PE makes the determination of which are primary and which secondary complicated. Indeed, the etiology of PE as well as the underlying causes of most associated alterations are unknown. Despite this, there are changes associated with PE that are sufficiently well established to allow for a meaningful discussion. Among these are changes in the handling of sodium in PE relative to these same processes in uncomplicated pregnancy. In this review evidence of altered cell sodium concentration will be explored with special attention given to the possibility that in the setting of PE a reduction in sodium pump (SP) activity leads to those changes. The further possibility that these alterations might be due to an endogenous SP inhibitor will also be considered.

3. SODIUM REGULATION IN UNCOMPLICATED PREGNANCY

Human pregnancy is characterized by a marked increase in cardiac output (~40-50%) and a consequent, proportional increase in the filtered fraction of sodium (1).

Moreover, there is an increase in the intravascular volume (30-50%) and some have speculated that this is achieved by increased sodium (Na) retention (2). Both of these changes may be related to the marked activation of the renin-angiotensin-aldosterone system (RAAS) in pregnancy. Despite this, there is some evidence to suggest that normal pregnancy represents a state of modest sodium wasting (3). The question of whether there is a programmed, primary increase in the RAAS that results in Na retention and volume expansion or whether there is a secondary activation of the RAAS is still debated. The factor(s) responsible for the RAAS activation is not fully known but might include the positive influence of estrogen on renin gene transcription (4).

Natriuretic factors also appear changed in pregnancy. Progesterone is markedly increased in pregnancy and opposes distal tubular reabsorption of sodium (5). Atrial natriuretic peptide (ANP) has been reported at higher circulating concentration in the latter half of normal pregnancy (6), but this is not a uniform finding (7). Dopamine has been little studied during pregnancy, but our work suggests that urinary dopamine output in women in sodium balance was somewhat higher during pregnancy than post partum (8). The digitalis-like factor, an endogenous sodium pump inhibitor and a putative natriuretic and possibly hypertensinogenic factor, has not been thoroughly studied in the setting of normal pregnancy, but some evidence suggests modest increases in its levels (2). Hence, while aldosterone enhances sodium retention, several natriuretic factors may oppose aldosterone's effects. Serum sodium concentration is modestly, but consistently, reduced during pregnancy, even with the markedly increased circulating aldosterone concentration (8).

4. SODIUM REGULATION IN PREECLAMPSIA

Many studies suggest that PE is accompanied by a reduced plasma volume when compared with uncomplicated pregnancy (9). Serum sodium concentration, despite potential differences in intravascular volume in PE, is indistinguishable from findings in uncomplicated pregnancies (10). Serum creatinine, as a crude measure of renal clearance, is reported as elevated (11) and glomerular filtration rate (GFR) markedly reduced in PE (12), suggesting renal changes beyond those related to proteinuria. Reduced GFR would contribute to the moderately reduced rate of sodium excretion in response to a saline infusion observed in PE (12).

Elements of the RAAS are clearly and substantially decreased in PE compared with uncomplicated pregnancy, and this is not related to sodium intake (13). The decreases in plasma aldosterone concentration are especially profound and could in theory contribute to the reduced plasma volume observed in PE (13).

Natriuretic factors also appear altered. Many studies report ANP as markedly increased in the setting of PE (14), but this is not a uniform finding (10). This increase may precede the clinical manifestations of disease (15). Such a change could in theory contribute to the reduced plasma volume, but AVP does not appear to mediate volume changes in PE (10). Indeed, the effects of ANP on the vasculature during pregnancy or PE are uncertain. Studies have demonstrated that the hypertension of PE is due to vasoconstriction (16) while ANP is a potent vasodilator. Moreover, ANP is also sensitive to volume and might be anticipated to be suppressed by the reduced intravascular volume of PE (15). Plasma dopamine has been not been extensively studied. One study found it elevated in PE women (17). The digitalis-like factor, as discussed later, may also be increased in the setting of PE.

In summary then, there are pronounced changes in both sodium retentive factors and natriuretic factors in PE. Neither set of changes would seem appropriate for the reduced volume status of PE and indeed both sets of factors are changed in ways that would favor reductions in the intravascular volume as seen.

5. CELL SODIUM REGULATION IN UNCOMPLICATED PREGNANCY

Multiple transport systems move sodium ions across the cell membrane. The primary sodium transport system is termed the (Na,K)ATPase or sodium pump (SP). It moves 3 sodium ions out of the cell for every 2 potassium ions moved in, maintaining the appropriate ionic milieu in the cytosol and giving rise to the cell membrane potential. Additionally, there are the sodium-lithium countertransport system (CTT) (which may or may not be related to the sodium-proton exchanger or antiporter, NHE) and the sodium and potassium cotransport system (COT). In some cells there is also a sodium-calcium exchanger (SCE). If other systems allow for excess Na to enter, the SP is activated to reestablish the appropriate ionic milieu. Consequently, other Na transport processes can indirectly affect the activity of the SP. It should be noted that if cell Na levels are increased, this requires reduced SP capacity.

5.1. Cell Sodium and Cell (Na,K)ATPase or Sodium Pump (SP) in Uncomplicated Pregnancy

In normal pregnancy most studies report an increase in erythrocyte SP activity (18-24) (typically measured by ^{86}Rb uptake into cells), lymphocyte Na efflux (25), platelet Na efflux (26), rubidium uptake into human umbilical vein endothelial cells incubated in autologous serum (27), an increase in erythrocyte sodium pump number (2,19,21,24,28) and a decrease in erythrocyte intracellular sodium ion concentration (2,20-23,25,28). Increased cell K concentration has been documented where studied (2,19,29). One study reported an increased reticulocyte count (30), reticulocytes having more SP units, but this appears to be variable (31). Moreover, the same changes are observed in blood cells other than erythrocytes.

What purpose this apparent change in SP number serves is unknown. It is also not established that peripheral blood cell changes reflect a more generalized increase in SP activity. There is some suggestion that a SP inhibitor may be present in normotensive pregnant women (2). While it is clear that one or more factors that crossreact

with digoxin or ouabain antibodies are markedly increased in pregnancy (32), these factors may not represent an active SP inhibitor (33). If such a factor were increased, SP number and possibly activity may be increased simply to offset their presence. However, the reduced intracellular Na concentration observed suggests that the increase in SP number and activity more than compensate for any such inhibitor even if increased. One hypothetical benefit of increased active sodium transport, with a consequent reduction in cell Na, would be a hyperpolarization of mechanically active cells, as in the vascular smooth muscle (VSM), which could reduce peripheral resistance and allow for greater blood flow to the fetal-placental unit. If such changes were present in the uterus, it would tend to reduce the contractile activity of that tissue and maintain it in a quiescent state. Indeed, the uterus is hyperpolarized during most of pregnancy.

The increase in SP abundance in normal pregnancy may be mediated transcriptionally. Aldosterone is known to upregulate SP mRNA levels in some cultured cells (34). Hence, increased serum aldosterone in pregnancy might be predicted to increase expression of SP units in several cell types, possibly peripheral blood cells. Progesterone may also increase SP mRNA, but this hasn't been studied in pregnancy (35). There is an indication of altered myometrial SP isoform protein abundance in human (36) and rat pregnancy (37).

5.2. Other Cell Sodium Transport Systems in Uncomplicated Pregnancy

Other cellular, sodium handling systems have been less extensively studied in uncomplicated human pregnancy. In very general terms there is evidence that ouabain-insensitive Na efflux (i.e. not SP mediated) is increased in erythrocytes in pregnancy (19,25,38). In the setting of normal pregnancy, there is consistent evidence that the maximal activity of the red cell CTT system is increased (29,39-43), but the implications of such a change are unknown. Indeed, the exact function of the CTT system is still debated (44). The status of the NHE in normal pregnancy is largely unstudied. One small study found no change in the NHE with uncomplicated pregnancy (45). COT has been reported by some to be increased in normal pregnancy (46,47) and by others to be decreased (29). Increased activity would favor movement of Na ion out of the cell. The cations are transported in conjunction with an equal number of anions, usually chloride, and hence the actions of the system do not modify the cell membrane potential. There appears to be no available work on the status of SCE in cells or tissues expressing that system in pregnant women.

6. CELL SODIUM TRANSPORT IN PREECLAMPSIA

The interest in sodium transport arises from the demonstration that increased vascular smooth muscle (VSM) sodium, brought about by SP inhibition, increases the sensitivity of the vascular to pressor substances (48). Moreover, if the cell sodium is sufficiently high, it leads to direct contraction of VSM (49). Such a heightened contractile state of the vasculature in response to elevated cell Na is mediated by a secondary increase in cytosolic, ionized calcium (50). As mentioned, the hypertension manifest in PE is a result of increased peripheral vascular resistance (16) and consequently increased cell sodium represents one of several candidate mechanisms to explain the increase in vessel tone and hypertension in PE.

6.1. Cell Sodium and Potassium in Preeclampsia

While studies of cell Na and K changes in normal pregnancy have been quite consistent, their status in the setting of PE is less clear. Most report significant increases in erythrocyte cell Na (38,51-57), lymphocyte cell Na (58), and leucocyte cell Na (54,58,59) in women having PE compared with normotensive pregnant women. Findings of no change in cell Na accompanying PE relative to normal pregnancy have also been reported (18,22,28,40,60,61). In a few other cases the results seemed intermediate with modest, but statistically insignificant increases in cell Na (19,62,63). Collectively, the studies do not allow for any firm conclusion regarding cell Na in PE, but more strongly support an increase in cell Na. Why are the results discrepant? The definition of PE in most studies seemed adequate to rule out the inclusion of women with chronic or gestational hypertension, where hypertensive mechanisms may be different. However, Macphail and coworkers found women with PE women in his study still had abnormally high blood pressures 20 weeks after delivery (22). One criterion for true PE has been a normalization of blood pressure post partum. Methodologies for cell handling and sodium assay vary, and it is likely that delays before processing cells or lengthy methods or prolonged or repeated washing of cells might dissipate a small excess in cell Na, especially given that the SP or COT systems cannot be inhibited without confounding the results. It is also conceivable that different populations, especially genetically different populations, actually have different mechanisms underlying their hypertension giving rise to discordant findings. For example, Sowers work has been primarily with African-American women (61). The majority of negative studies were from the United Kingdom and may reflect a unique population (18,22,28,60). However, the racial or genetic composition of women for most of these studies was not detailed leaving this an open question. Hence, the hypothesis relating cell Na and hypertension remains suggestive, but unconfirmed in PE. The study of cell K levels in PE has been less frequent. In general those studies finding no change in cell Na also found no change in cell K and those studies finding an increase in cell Na found a decrease in cell K.

6.2. Cell (Na,K)ATPase or Sodium Pump (SP) in Preeclampsia

SP status in women with PE is something of an open question. The assay of the SP has been performed variously and has undoubtedly contributed to contradictory results. A major issue is the possible presence of a circulating SP inhibitor in pregnant women that is significantly increased in PE (63, discussed in detail hereafter). While this is still controversial in PE (64), such an increase in a SP inhibitor is well evidenced in essential hypertension, volume-expanded forms of secondary

hypertension (e.g. acromegaly) and experimental animal hypertension (e.g. DOCA-salt, reduced renal mass hypertensive models) (65-67). Current data support more than one such factor being present in the circulation and that these factors bind tightly and specifically to the digitalis cardioglycoside binding site of the SP causing inhibition (68-70). If we accept that such inhibitors are present and potentially increased in PE, a central issue then is whether the effect of the inhibitor is preserved during the assay, maintaining diminished SP activity (and increased cell Na). Conversely, has this factor been partially or completely displaced in the processing of cells, resulting in no apparent change or even an increased number of active SP units with consequent higher activity? Again, assay methods that are time consuming, involve many washing steps, use high ionic strength buffers, or are done without close monitoring of pH are more likely to disrupt the binding of a SP inhibitor from the SP. Methods for the assessment of the SP have included determination of the rate of rubidium uptake into peripheral blood cells, placental cells or umbilical artery cells with or without a sodium load, the measurement of ^{22}Na efflux after loading cells, the assay of the residual ATPase activity of cell membrane ghosts, or the determination of the number of SP units measured by ^{3}H-ouabain binding. This multiplicity of approaches and the absence of a way to account for the influence of a SP inhibitor in most has undoubtedly contributed to inconsistency in the findings.

Nevertheless, a majority of studies have found significant reductions in SP activity or SP unit number accompanying PE as manifest in erythrocytes (29,52,53,56,57,71-75; In Reference 71 the decreases were significant for cord erythrocytes from preeclamptic pregnancies and lower but not statistically significantly so for erythrocytes from the preeclamptic mothers), leucocytes (59), platelets (26), umbilical vein endothelial cells (27) and placental cells (76). Increases in SP activity in PE have been reported in erythrocytes (18 (but criteria for PE appear inadequate),19,38) or lymphocytes (25). Didden (77) found no difference in active erythrocyte Na transport in women who developed PE. Kaplay (62) in a small study found no significant differences in erythrocyte SP activity comparing women with PE to third trimester women with edema as the control group. Poston incubated normal peripheral leucocytes in the serum of 19 women with PE with gestational ages of 28-40 weeks and found no change in SP function after the incubation (64). Aronson studied digoxin binding and rubidium uptake in erythrocytes from 18 women with PE compared with gestationally age-matched controls and found no significant differences (28).

If significantly lower and potentially higher SP activity (as well as increased cell Na) can both be indicative of the influence of a SP inhibitor, then one might interpret a majority of studies being consistent with a SP inhibitor in PE. This remains an intriguing possibility. Importantly, one study measured both ouabain binding, to tally the number of SP units, and rubidium uptake and found increased SP sites but significantly reduced activity (76). This pairing of approaches provided more information and argued more strongly for the imprint of a SP inhibitor on the system. Developing standardized assay conditions and methods that clearly displace such inhibitors or insure their continued binding to the SP would be exceptionally valuable in answering the question at hand and should be possible. Some effort has been made in this regard (78).

Study of the actual expression of the SP α-isoform (the functional unit of the SP) protein or mRNA in PE has been very limited, but in one study there appeared to be decreases in both mRNA and protein expression for the α2 isoform of the SP in myometrium and a reduction in mRNA of the α1 and α2 isoforms without reductions in the placental membrane protein expression of the two isoforms in PE (36). This study also suggested that gestational age may be a very important confounding variable for the evaluation of the SP.

Taken together, there is a suggestion that SP activity is reduced in PE. Some of this reduction may be due to increased levels of a SP inhibitor, which may be elevated in the circulation of women with PE. Alternatively, the reductions may reflect decreased expression of one or more isoforms of the SP. In some cases this loss of SP number and ion transport capacity may result in a modest increase in cell Na. Even small decreases in cell SP activity can have substantial physiologic effects (49). Hence, the actual changes in SP activity may be small and yet pathologic. Patient to patient variability may be substantial and cross sectional studies may be hard to interpret if there are changes in SP number and activity as a consequence of gestational age (indeed there is some evidence in animals for a gestational age related increase in the SP isoforms (37)). Prospective, longitudinal studies are difficult to carry out, but are clearly needed to resolve these questions in a more definitive manner. Finally, and perhaps most problematic, not one study to date has actually investigated the relevant tissue, i.e. the maternal vasculature.

6.3. Other Cell Sodium Transport Systems in Preeclampsia

While red cell CTT is increased in normal pregnancy, some studies have reported yet higher levels of CTT in PE (40,43). Several other reports have found no difference in red cell CTT between normotensive, uncomplicated pregnant women and those with PE or gestational hypertension (29,41,42,63). In our hands the red cell CTT was significantly elevated in women with gestational hypertension (63). Those with PE had intermediate, and non-significantly higher CTT than normal pregnant women in one study (63) but significantly elevated CTT in PE women in another (43). There are some potentially confounding variables. One might be the assay itself. Not only is it technically demanding, the measurement determines movement from a high Na environment to a Na free environment and the choice of the Na substitute may yield different results (44). African American women are known to have significantly lower erythrocyte CTT than Caucasian subjects (79). Consequently, studies combining black women with historical origins in Africa and Caucasian women may eliminate differences present in either group alone. Type I

diabetic pregnant women without hypertension appear to have slightly higher red blood cell CTT than non-diabetic pregnant women (43). Hypertensive Type I diabetic pregnant women with proteinuria appear to have CTT very similar to the non-hypertensive diabetic pregnant women and to be lower than PE. Consequently, the make up of the study population may increase inconsistency.

The use of red cell CTT has been primarily as a marker and it has been demonstrated in several groups' work to be associated with hypertension and perhaps more particularly with Syndrome X, which is a manifestation of both insulin resistance and hypertension (80). Elevated CTT has also been associated with risk for certain complications of diabetes (81). It is of interest that PE is also associated with insulin resistance (82).

The study of the NHE in the setting of PE has been limited. There has been one published study carried out on placentas taken from either normotensive or preeclamptic pregnancies. Activity was not measured but the expression of NHE protein was found to be decreased in PE (83). Those authors speculated that this might impair water and/or electrolyte uptake into this tissue. One other study found increased NHE activity in erythrocytes from PE women (45). There is one report of significantly elevated erythrocyte COT in women with PE compared to either non-pregnant or normotensive pregnant women (29). There is a second report finding exactly the opposite with the erythrocyte COT being significantly reduced in PE (46). There is a single report of human umbilical artery SCE being depressed in women with PE compared to normotensive, uncomplicated pregnancies (84). However, increased SCE is thought to increase muscle contraction and hence this change if confirmed would oppose increased VSM contraction in placental arteries in these women. Clearly, it is premature to draw conclusions regarding these transport systems in PE.

7. SODIUM PUMP INHIBITORS IN UNCOMPLICATED PREGNANCY

Studies have shown increases in factors present in human serum that cross react with anti-digoxin antibodies. Several studies suggest that levels of these digoxin-like immunoreactive factors increase dramatically over the course of pregnancy (32,33,64,85,86). However, the free levels of these factors in pregnancy may be much less affected (33,87). Moreover, SP activity increases in uncomplicated pregnancy in comparison with the non-pregnant or post partum state even though there may be modest increases in levels of an active SP inhibitor as mentioned (33).

8. SODIUM PUMP INHIBITORS IN PREECLAMPSIA

As many as 100 studies of essential and volume-dependent forms of secondary hypertension demonstrate elevated levels of a factor(s) that inhibits (Na,K)ATPase activity and/or SP ion transport (65-67,88,89). Where studied, the circulating levels of these factors correlate with blood pressure (88,89). An equally impressive number of studies have been done in animal models of hypertension with comparable results (90,91). However, the possible involvement of a SP inhibitor in PE has been less well studied. There are several studies that report increased levels of a SP inhibitor as measured by digoxin antibody (64,93-102), ouabain antibody (103), marinobufogenin antibody (103), by inhibition of SP (26,27,29,52-57,59,71-76) in women with PE. At the same time there have been other studies finding no evidence for increased levels of such an immunoreactive species (63,85,104,105) or of a SP inhibitor (28,62,64,77). One of the most provocative set of results has been provided by the use of anti-digoxin antibodies to treat PE. Digibind® is a anti-digoxin antibody Fab fragment used to treat overdoses of digoxin (106). However, on a few occasions its use has been extended to treat women with severe PE based on the possibility of a SP inhibitor mediating the hypertension of PE (107-109). In these cases there was remarkable success in lowering blood pressure for several hours (107-109). The Fab fragment has been used in hundreds of individuals without eliciting an immune response and the antibody has almost no affinity for other steroidal compounds, consequently, in those few cases, its ability to lower blood pressure suggests strongly that it binds a hypertensinogenic, digitalis-like factor.

9. CONCLUSION AND PERSPECTIVE

Existing research points to sets of changes in Na handling during normal pregnancy with some confidence: Serum Na concentration is significantly reduced during normal pregnancy and there is a profound, progressive activation of the renin-angiotensin-aldosterone system during the course of pregnancy. Over this same interval there is an increase in SP number and activity and a reduction in cell Na concentration manifest in peripheral blood cells with similar findings in a few studies of tissue or tissue cells. Erythrocyte CTT is also increased.

In PE the RAAS is markedly reduced compared with uncomplicated pregnancy. Natriuretic factors may also be changed with evidence for an increase of the atrial natriuretic factor. Changes in cell Na transport likely accompany PE. The strongest and most consistent findings indicate that there is an increase in cell Na, a decrease in cell SP activity and higher levels of a digitalis-like SP inhibitor in PE. Though limited, there are even studies that use an antibody fragment targeting such inhibitors to treat effectively the hypertension of PE. One must also acknowledge that several studies did not find these changes and this precludes the current body of work being definitive. Other Na handling systems were less frequently studied and the results more contradictory.

Improvements in techniques are needed, in particular the understanding and control of variables that might affect the assay outcome. The simultaneous application of several methods holds promise of more informative studies. There is enough evidence drawn from the several studies considered here to make likely that alterations in Na transport accompany PE and may even

contribute to the hypertension of PE which remains one of the most problematic complications of this disease. While the antecedents of the hypertension are critical for an understanding of PE, the factors that drive the blood pressure are also of substantial importance and are consequently deserving of far more study.

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