Erythrocyte cation fluxes during the menstrual cycle in normal female subjects

J. C. MONAGHAN¹, D. A. WILLCOCKS¹, M. J. SINOSICH² AND G. S. STOKES¹
Hypertension Unit, ¹Department of Clinical Pharmacology and ²Department of Obstetrics and Gynaecology, Royal North Shore Hospital, St. Leonards, N.S.W., Australia

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SUMMARY

1. Studies of erythrocyte cation transport mechanisms in vitro were performed on eight normotensive, premenopausal female subjects at the mid-points of the follicular and luteal phases of their menstrual cycles. Concurrent plasma concentrations of 17β-oestradiol, progesterone, aldosterone and renin activity were measured.

2. Ouabain-resistant, frusemide-resistant rubidium influx (an index of passive potassium diffusion) was significantly lower in the luteal than the follicular phase.

3. In further studies in four of the eight subjects, the mean rate constant of the rubidium influx measurement was also lower in the luteal than in the follicular phase.

4. There were no changes in Na⁺-K⁺ co-transport, sodium pump activity or intracellular cation concentrations throughout the cycle.

5. There was a tenfold fall in the mean plasma 17β-oestradiol/progesterone ratio, as well as increases in plasma aldosterone concentration and renin activity between the mid-follicular and mid-luteal phases.

6. We conclude that changes in plasma oestrogen/progesterone ratio during the menstrual cycle may be associated with alterations in passive potassium diffusion.

Key words: Cation transport, erythrocytes, menstrual cycle.

Abbreviations: MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; PRA, plasma renin activity.

INTRODUCTION

Erythrocyte sodium and potassium transport mechanisms show differences between the sexes [1-4], and are altered by oral contraceptives [5-9] or pregnancy [9-11]. This strongly suggests the influence of hormonal factors. Hormonal changes associated with the menstrual cycle are well documented [12-15]. During the luteal (post-ovulatory) phase of the menstrual cycle, the plasma concentration of progesterone is higher than during the follicular (post-menstruation) phase. Progesterone is known to have a natriuretic effect [16] which leads to a rise in plasma renin activity (PRA) [17, 18] and plasma aldosterone concentration [17]. Oestrogen (17β-oestradiol and oestrone) concentrations show a biphasic pattern during the menstrual cycle with ovulatory and mid-luteal peaks [12, 13]. However, changes in erythrocyte cation transport fluxes during the menstrual cycle have not been fully investigated.

Therefore, we studied erythrocyte cation transport mechanisms to determine whether there are short term changes in sodium and/or potassium influx and intracellular electrolyte concentrations between the mid-follicular and mid-luteal phases. Furthermore, we investigated relationships between erythrocyte cation transport and levels of circulating hormones. The possibility that transport flux changes may be related to changes in electrolyte balance was also examined.

SUBJECTS AND METHODS

Eight normotensive females (age range 27-42 years) with no family history of hypertension, taking no medication (including oral contraceptives) and with regular menstrual cycles were studied. Blood (85 ml) was collected from an antecubital vein between 09.00 and 10.00 hours on each of two separate occasions, 6 weeks apart, coinciding with the mid-follicular phase (days 6-10) of the menstrual cycle or the mid-luteal phase (days 21-23) in random order. This protocol was adopted to minimize haematological changes due to repeated blood sampling. Twenty-four hour collections of urine were made for measurement of electrolytes and creatinine to ensure that any changes
observed in cation transport were not due to changes in dietary sodium or potassium. Three consecutive blood pressure measurements (taken in the seated position) were made by the same observer using a standard cuff and sphygmomanometer. Body weight was also measured. All subjects gave informed consent to the study, which had institutional approval.

Measurements in vitro included erythrocyte potassium influx as indicated by rubidium (⁸⁶Rb⁺) influx [19] and sodium (³²Na⁺) influx, mean corpuscular volume (MCV) and haemoglobin concentration (MCHC), reticulocyte count, plasma electrolytes and creatinine, and erythrocyte electrolyte (Na⁺ and K⁺) concentrations.

In order to determine Na⁺ and Rb⁺ influxes, the plasma and buffy coat were firstly separated from erythrocytes at 4°C by centrifugation. A modification of the method of Woods et al. [20] was used to measure Rb⁺ influx. Cells were triple-washed at 4°C and then incubated for 1 h at 37°C in an artificial medium containing (mmol/l): 144 Na⁺, 5 Rb⁺, 1.34 Ca²⁺, 1.26 Mg²⁺, 131 Cl⁻, 18.7 HPO₄²⁻ and 5.58 glucose, pH 7.4. Net Na⁺ influx was measured in cells triple-washed at 4°C in an artificial medium containing (mmol/l): 145 Na⁺, 5 K⁺ and 20 imidazole chloride, pH 7.4, and then incubated for 20 min at 37°C in the same medium containing ²²Na⁺ (1 μCi/ml) and 10 mmol/l glucose [21]. Total osmolalities of the two influx media were 298 mosmol/kg and 306 mosmol/kg, respectively. Both influx rates were expressed per litre of cells by use of MCHC and cyanmethaemoglobin concentration after lysis of the cells [21]. To measure the various components of the fluxes, ouabain and frusemide were added to the incubation media to give final concentrations of 0.1 mmol/l and 1 mmol/l, respectively. Sodium pump activity (ouabain-sensitive Rb⁺ influx) was calculated as the difference in Rb⁺ influx after incubation with and without ouabain; Na⁺–K⁺ co-transport (ouabain-resistant, frusemide-sensitive Rb⁺ influx) was calculated as the difference in Rb⁺ influx between that in the presence of ouabain (ouabain-resistant Rb⁺ influx) and in the presence of both ouabain and frusemide (ouabain-resistant, frusemide-resistant Rb⁺ influx). The ouabain-resistant, frusemide-sensitive component of Na⁺ influx also represents Na⁺–K⁺ co-transport while the ouabain- and frusemide-resistant component of Na⁺ influx represents passive Na⁺ diffusion and Na⁺–Na⁺ exchange.

Intracellular electrolytes in lysed cells [22] were measured using a Corning 405 flame photometer and results were corrected for MCHC and cyanmethaemoglobin concentrations and expressed as mmol per litre of cells. Intra- and inter-assay coefficients of variation for fluxes and intracellular Na⁺ and K⁺ concentrations have been previously reported [6].

PRA was measured by a modification of the method of Huber et al. [23]. ¹²⁵I radioimmunoassays were used to measure plasma levels of various hormones: aldosterone (International CIS, France), 17β-oestradiol (R.I.S., Germany) and progesterone (Farnos Diagnostica). Urinary electrolytes were determined by flame photometry and urinary creatinine by the method of Edwards & Whyte [24]. Plasma electrolytes were measured by autoanalyzer techniques. Results are expressed as mean ± SEM. Statistical analyses were by paired t-test and linear regression correlations. P < 0.05 was taken as the level of statistical significance.

## RESULTS

There were no significant differences in blood pressure, body weight, MCV, MCHC, plasma Na⁺ or K⁺ concentrations, or urinary excretion of Na⁺, K⁺ or creatinine between the phases of the menstrual cycle, in the eight subjects studied. All values were within normal ranges. All subjects were considered to have ovulated normally, since the plasma progesterone concentration exceeded 20 nmol/l at the time of the luteal phase sampling [25]. Mean plasma progesterone concentration rose from 4.0±0.8 nmol/l in the mid-follicular phase to 53.3±6.4 nmol/l

### Table 1. Intracellular cation concentration, Na⁺ influx and Rb⁺ influx components at mid-follicular and mid-luteal phases of the menstrual cycle

<table>
<thead>
<tr>
<th></th>
<th>Folicular phase (n = 8)</th>
<th>Luteal phase (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intracellular cation concentration (mmol/litre of cells)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na⁺</td>
<td>8.8 ± 0.4</td>
<td>8.6 ± 0.4</td>
</tr>
<tr>
<td>K⁺</td>
<td>97.8 ± 1.4</td>
<td>97.3 ± 2.2</td>
</tr>
<tr>
<td><strong>Rb⁺ influx (mmol h⁻¹ litre⁻¹ of cells)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1.80 ± 0.08</td>
<td>1.75 ± 0.08</td>
</tr>
<tr>
<td>Ouabain-resistant</td>
<td>0.45 ± 0.06</td>
<td>0.40 ± 0.02</td>
</tr>
<tr>
<td>Ouabain-sensitive</td>
<td>1.35 ± 0.04</td>
<td>1.36 ± 0.07</td>
</tr>
<tr>
<td>Ouabain-resistant, frusemide-sensitive</td>
<td>0.33 ± 0.06</td>
<td>0.33 ± 0.03</td>
</tr>
<tr>
<td>Ouabain-resistant, frusemide-resistant</td>
<td>0.11 ± 0.01</td>
<td>0.06 ± 0.01*</td>
</tr>
<tr>
<td><strong>Na⁺ influx (mmol h⁻¹ litre⁻¹ of cells)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1.82 ± 0.08</td>
<td>1.88 ± 0.04</td>
</tr>
<tr>
<td>Ouabain-resistant, frusemide-sensitive</td>
<td>1.53 ± 0.05</td>
<td>1.59 ± 0.03</td>
</tr>
<tr>
<td>Ouabain-resistant, frusemide-sensitive</td>
<td>0.29 ± 0.03</td>
<td>0.29 ± 0.03</td>
</tr>
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Results are given as means ± SEM. Statistical significance: *P < 0.02.
(P < 0.001) in the mid-luteal phase. PRA showed a corresponding significant increase from 1.5 ± 0.3 to 3.4 ± 0.6 ng of angiotensin I h⁻¹ ml⁻¹ (P < 0.005) as did plasma aldosterone concentration which rose from 193 ± 20 to 290 ± 31 pg/ml (P < 0.02). The rise in mean plasma 17β-oestradiol concentration between the two phases (270 ± 55 versus: 318 ± 38 pmol/l) was not statistically significant as one patient showed a decrease. However, the plasma 17β-oestradiol/progesterone ratio decreased from 0.071 ± 0.012 in the mid-follicular phase to 0.007 ± 0.001 (P < 0.001) in the mid-luteal phase. Follicular and luteal phase plasma progesterone concentrations correlated significantly with plasma aldosterone concentrations (r = 0.71, P < 0.005) and PRA (r = 0.70, P < 0.005).

Table 1 shows the values for total Rb⁺ and Na⁺ influxes and their various components during the menstrual cycle. Ouabain-resistant, frusemide-resistant Rb⁺ influx (passive Rb⁺ diffusion) was significantly lower in the luteal phase than in the follicular phase (P < 0.02). There were no changes observed in any of the other Rb⁺ and Na⁺ flux components, nor in intracellular Na⁺ and K⁺ concentrations, during the cycle. The decrease in passive Rb⁺ diffusion was not associated with variations in MCHC, MCV, or plasma electrolytes between the phases, nor was it directly associated with changes (differences between follicular and luteal phases) in plasma progesterone, 17β-oestradiol, aldosterone or PRA. However, a significant relationship was found between the plasma 17β-oestradiol/progesterone ratio and passive Rb⁺ diffusion for follicular and luteal values (r = 0.70, P < 0.005).

In four subjects, mid-follicular and mid-luteal values for passive Rb⁺ diffusion were re-determined at external Rb⁺ concentrations ranging from 3 μmol/l to 10 mmol/l. As expected, passive Rb⁺ diffusion was a linear function of external Rb⁺ concentration in both the follicular and luteal phases of the menstrual cycle. The mean rate constant was significantly less in the mid-luteal phase than in the mid-follicular phase (0.017 ± 0.001 h⁻¹ versus 0.013 ± 0.001 h⁻¹, P < 0.025).

DISCUSSION

Our previous studies of erythrocyte cation transport have shown that inward Na⁺−K⁺ co-transport is significantly greater in men than in women not taking oral contraceptives [6], confirming the findings of Duhm et al. [1]. We also demonstrated that women taking oral contraceptives had higher values for the Na⁺−K⁺ co-transport component of Rb⁺ influx than controls [6]. Other groups have shown sex differences in Na⁺−Li⁺ countertransport [1, 3, 4]. Also, Na⁺−Li⁺ countertransport [7, 26], outward Na⁺−K⁺ co-transport [8, 26] and the active sodium pump [26] are altered by oral contraceptives. These studies suggest that there are hormonal influences upon erythrocyte transport mechanisms, without indicating which hormone or hormones might be responsible.

In view of the known hormonal changes which take place during the menstrual cycle, we investigated the effect of these changes on erythrocyte sodium and potassium transport mechanisms. Using techniques for measuring sodium and rubidium influxes in erythrocytes in vitro, we have been able to show a significant decrease in the inward passive diffusion of Rb⁺ during the luteal phase of the menstrual cycle compared with the follicular phase. Passive Rb⁺ diffusion was shown also to be directly related to the plasma 17β-oestradiol/progesterone ratio.

As expected, plasma progesterone concentration was significantly higher in the luteal phase than in the follicular phase. In agreement with other studies [17, 18] there was also a significant increase in PRA and plasma aldosterone concentration during the luteal phase. That this stimulation of the renin–aldosterone axis was due to increased progesterone production was suggested by the direct relationships obtained between progesterone levels and PRA and/or plasma aldosterone concentration. These findings are in agreement with other published data [18], which demonstrate that progesterone has an effect on PRA and antagonizes aldosterone at the renal tubular level.

We found a linear relationship between passive Rb⁺ diffusion and external Rb⁺ concentration, thus verifying the specificity of the method used for measuring passive diffusion [27]. Since the passive and active movements of Rb⁺ ions across the cell membranes reliably indicate those of K⁺ [27, 28], our findings suggest that there is a decrease in K⁺ permeability during the luteal phase. This finding contrasts with that of Gallery et al. [29], who reported that there was no significant difference in passive Rb⁺ influx between the follicular and luteal phase. The discrepancy between results is unexplained, but might reflect the earlier luteal sampling time in their study, which was days 15–18 of the cycle, compared with days 21–23 in the present study.

The lack of changes in intracellular K⁺ concentration, sodium pump activity and Na⁺−K⁺ co-transport in our study are in agreement with the findings of Gallery et al. [29] and M’Buyamba-Kabangu et al. [30]. In contrast, Krzesinski et al. [8] showed a decrease in outward Na⁺−K⁺ co-transport in the follicular phase of the menstrual cycle. This was claimed to be due to an oestrogenic effect. However, the observation was unsubstantiated since no data was presented as to the levels of hormones, such as 17β-oestradiol, in their subjects. Also, outward co-transport was measured, not inward as in the present study, and the two are not necessarily correlated [31].

In conclusion, hormonal changes during the menstrual cycle do not affect erythrocyte cation transport in the same way as oral contraceptives. This may be because of differences in molecular structure between the exogenous and endogenous hormones, or because the effects of contraceptive hormones on the erythrocyte membrane involve new protein synthesis and the replacement of circulating erythrocytes with a new generation of cells from nucleated precursors. However, a minor change in passive permeability of the erythrocyte membrane was found between the mid-follicular and mid-luteal phases.
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