Catamenial variations in erythrocyte sodium–lithium countertransport and blood pressure

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1. We undertook a temporal study of external sodium-stimulated lithium efflux (sodium–lithium countertransport) in erythrocytes and blood pressure by measuring these two parameters in three phases of the menstrual cycle (menstrual, midcycle and luteal phases) in 22 healthy, non-medicated females with regular menstrual cycles. Plasma oestradiol and progesterone levels were also determined.

2. Sodium–lithium countertransport activity (activity in 140 mmol/l external NaCl) in the midcycle phase (0.176 ± 0.017 mmol h⁻¹ l⁻¹ of cells) was lower than in the menstrual (0.192 ± 0.016 mmol h⁻¹ l⁻¹ of cells, P < 0.030) and luteal (0.203 ± 0.018 mmol h⁻¹ l⁻¹ of cells, P < 0.030) phases. The Vₘₐₓ of the transporter changed similarly but the Kₘₜ was unaltered.

3. The plasma oestradiol level was 628.9 ± 39.1 pmol/l in the midcycle phase, higher than in the menstrual (232 ± 18.5 pmol/l, P < 0.001) and luteal (372.5 ± 28.1 pmol/l, P < 0.001) phases. The progesterone level was 28.6 ± 2.1 nmol/l in the luteal phase, and values were lower in the menstrual (2.5 ± 0.3 nmol/l, P < 0.001) and midcycle (2.8 ± 0.4 nmol/l, P < 0.001) phases.

4. There was no correlation between plasma oestradiol and sodium–lithium countertransport activity or Vₘₐₓ during the menstrual cycle, but plasma progesterone was positively correlated with sodium–lithium countertransport activity (r = 0.478, P < 0.025, n = 22) and Vₘₐₓ (r = 0.551, P < 0.045, n = 14) in the luteal phase.

5. Systolic blood pressure did not change significantly during the menstrual cycle. However, the diastolic pressure showed variation similar to that in sodium–lithium countertransport activity/Vₘₐₓ, its midcycle value of 66.6 ± 1.4 mmHg being lower than that in the luteal (71.6 ± 1.3 mmHg, P < 0.025) and menstrual (70.6 ± 1.4 mmHg, P < 0.025) phases.

6. We conclude that sodium–lithium countertransport activity exhibits catamenial variation. Therefore we suggest, given this observation, that blood sampling for the assessment of the state of activity of the transport system be standardized in relation to a phase of the menstrual cycle in future studies involving females.

INTRODUCTION

The possible influence of gender on the external sodium-stimulated lithium efflux, otherwise known as sodium–lithium countertransport (SLC), was mooted by Greil et al. [1] and subsequently reported by others [2]. Further evidence was provided by Cooper et al. [3], who found, among college students, that SLC was about 29% higher in males aged 19.9 ± 2.5 years (mean ± SD; n = 25) than in females aged 20.7 ± 2.9 years (n = 12). Against the background of the reported association between high SLC activity and hypertension [4], this greater activity of the transporter in males was regarded by Cooper et al. [3] as being consistent with the greater prevalence of hypertension among men. Similar gender-related differences in SLC have been observed in both normotensive and hypertensive individuals by most [5–7] but not all [8] workers in other larger studies that included older age groups.

Available evidence suggests that gender-related difference in SLC activity is multifactorial, and regular intake of moderate amount of alcohol by males has been incriminated as one factor [7]. SLC is similar in both sexes in childhood, and the sex-related difference in the activity of the transport system, most pronounced in the reproductive years of females, is attenuated to insignificance from about 65 years of age [6, 7]. In females, pre- and post-pubertal SLC values are comparable. The same cannot be said of males in whom the transporter shows greater activity after puberty [7]. On the basis of these observations, it could be suggested that the significantly higher activity of SLC in post-pubertal males than in age-matched females is possibly due

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Abbreviation: SLC, sodium–lithium countertransport.
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to the increase in testosterone levels associated with that phase of development in males.

A very distinct physiological phenomenon that characterizes the reproductive years in females is the cyclical (monthly) variations in plasma oestrogen and progesterone levels. Compared with this cyclical change in females, the plasma testosterone level in men in their reproductive years is more stable. Consequently, assessment of a possible modulatory effect of testosterone on SLC in men in their reproductive years is not likely to be critically dependent on the timing of the SLC assay. However, the same cannot be said of similar study in females. Plasma oestrogen and progesterone levels vary significantly during the menstrual cycle, and, in view of the well-documented susceptibility of the transport system to acute modulation by exogenous/endogenous factors [9, 10], correct and accurate evaluation of the possible effect(s) of these hormone on SLC will entail measuring the activity of the transporter at definite times during the menstrual cycle, for example, the menstrual, midcycle and luteal phases. Our preliminary observations suggest that SLC activity changes during the menstrual cycle [11]. In the present study we evaluate this possibility in greater detail and in relation to blood pressure.

**MATERIALS AND METHODS**

Twenty-two non-medicated females with regular menstrual cycles were recruited. All subjects participated after giving their informed consent and Ethics Committee approval was obtained. They were aged 30.7 ± 1.8 years (mean ± SEM) and weighed 64.2 ± 1.7 kg (mean ± SEM). Each was studied in the morning on three occasions, namely days 1–4 of menstrual flow (menstrual phase), presumed time of ovulation (midcycle phase) and 7–10 days thereafter (luteal phase). At each visit, blood pressure in the sitting position was determined as the average of three readings made, after 10 min rest, with a Hawksley random zero sphygmomanometer. Blood samples were also taken for SLC, serum oestradiol and serum progesterone determinations. Assay of SLC in 14 subjects was performed as described previously [12], but only the one-point assay in 140 mmol/l NaCl was performed on samples from the remaining eight subjects. In order to assess reproducibility, repeat measurements of SLC activity (assay of the transport system in 140 mmol/l NaCl) in the menstrual cycle were made in three of the subjects 8 months to 1 year after the initial assay. Plasma levels of both oestradiol and progesterone were determined by radioimmunoassay method with kits from Diagnostic Product Corporation.

Data are given as means ± SEM, and differences during the menstrual cycle were assessed by analysis of variance. Between-phases comparison was by paired t-test with Bonferroni modification. For data not normally distributed, the Kruskal–Wallis test was used to evaluate differences during the experimental period. In all cases the minimum level of significance was set at \( P < 0.05 \).

**RESULTS**

**Timing of ovulation**

Two criteria were used to determine the correctness of the timing of ovulation: the start of the next menstrual period, which was 13–15 days after the presumed timing of ovulation in those who were asked \( (n = 16) \), and/or a higher oestradiol level compared with that in the other two phases (the menstrual and luteal phases).

**Hormone levels**

The midcycle oestradiol level was 628.9 ± 39.1 pmol/l, higher than that in the luteal (372.5 ± 28.1, \( P < 0.001 \)) and menstrual (232 ± 18.5, \( P < 0.001 \)) phases of the cycle. The level in the luteal phase was higher than that in the menstrual phase (\( P < 0.005 \)). Each of these values was within the normal range for the respective phase of menstrual cycle, namely 420–1500 pmol/l (midcycle), 140–1000 pmol/l (luteal) and 140–750 pmol/l (menstrual). Expectably, the highest progesterone level (28.6 ± 2.1 nmol/l) was in the luteal phase. Plasma levels of the hormone were lower in the menstrual (2.5 ± 0.3 nmol/l, \( P < 0.001 \)) and midcycle (2.8 ± 0.4 nmol/l, \( P < 0.001 \)) phases. All these values were also within the normal ranges of 12–89 nmol/l (luteal phase) and 0.3–4.8 nmol/l (menstrual and midcycle phases).

**SLC activity and kinetic parameters**

SLC activity in the luteal phase \((0.203 ± 0.018 \text{ mmol h}^{-1} \text{l}^{-1} \text{ of cells})\) was higher than in the midcycle phase \((0.176 ± 0.017 \text{ mmol h}^{-1} \text{l}^{-1} \text{ of cells}, P < 0.030, n = 22)\) and the menstrual phase \((0.192 ± 0.016, P < 0.030)\) (Fig. 1a). The same pattern of change was evident over 8 months to 1 year in the three subjects who allowed a repeat study over that period (Table 1). In the 14 subjects so studied, the \( V_{\text{max}} \) of the transporter changed similarly (Fig. 1b), but the affinity of the transporter for external sodium, \( K_m \), remained unaltered with values of 98.5 mmol/l (median, range 35–157 mmol/l), 89.5 mmol/l (37–172 mmol/l) and 92.5 mmol/l (38–164 mmol/l) in the menstrual, midcycle and luteal phases, respectively.

There was a positive correlation between plasma progesterone level and SLC activity \((P < 0.025, n = 22)\) and \( V_{\text{max}} \) \((P < 0.045, n = 14)\) in the luteal but not in the other two phases of the cycle (Figs 2a and 2b) (Table 2). However, neither of these parameters was related to the plasma oestradiol level during the study period (Table 2).
Blood pressure

Systolic pressure in the menstrual phase was 111.8 ± 2.0 mmHg, and was comparable with that in the midcycle phase (109.0 ± 1.9 mmHg) and the luteal phase (112.4 ± 2.1 mmHg). However, the midcycle diastolic pressure of 66.6 ± 1.4 mmHg was lower than that in the luteal phase (71.6 ± 1.3 mmHg, \( P < 0.025 \)) and the menstrual phase (70.6 ± 1.4 mmHg, \( P < 0.025 \)).

DISCUSSION

Menstrual cycle and SLC

Sex-related differences in some erythrocyte cation-transport systems are well documented. The activity of the sodium/potassium pump is higher in males than females [13], and so is that of the sodium/potassium co-transport [14, 15]. The implied possible modulatory role of sex hormones is supported by the findings that oral contraceptives medication is associated with an increase in ouabain-binding sites and sodium/potassium pump activity [16] and sodium/potassium co-transport [14, 15, 17].

The results of this study indicate a catamenial variation in SLC activity, the lowest (0.176 ± 0.017 mmol h\(^{-1}\) 1\(^{-1}\) of cells) and the highest (0.203 ± 0.018 mmol h\(^{-1}\) 1\(^{-1}\) of cells) values being at about the time of ovulation and in the luteal phase respectively (Fig. 1a). Similar observations have been made on other cation-transport systems. For example, lower sodium/potassium co-transport activity (197 ± 83 μmol h\(^{-1}\) 1\(^{-1}\) of cells) has been noted in the oestrogenic phase of the menstrual cycle than in the progestative (luteal) phase (426 ± 98 μmol h\(^{-1}\) 1\(^{-1}\) of cells) [18]. The earlier reported lack of catamenial changes in sodium/potassium pump activity [15, 19] has been contested by Webb et al. [20], who found that the activity of the pump was higher during the luteal phase than during the follicular phase in ten healthy women.

In the present study, the timing of the highest SLC activity coincided with the phase of menstrual cycle when the level of oestrogen is normally high and that of progesterone maximum (Fig. 1a). The reality and the consistency of such cyclical variation in SLC activity is supported by the fact that the same temporal changes were evident in repeat measurements made 8 months to 1 year later in the three subjects so studied (Table 1). An implication

<p>| Table 1. SLC activity at different stages of the menstrual cycle in three healthy subjects initially (I) and 8 months to 1 year later (II) |
|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Subject</th>
<th>Menstrual phase</th>
<th>Midcycle phase</th>
<th>Luteal phase</th>
<th>Menstrual phase</th>
<th>Midcycle phase</th>
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<td>1</td>
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<td>0.124</td>
<td>0.082</td>
<td>0.148</td>
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<td>0.196</td>
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<td>3</td>
<td>0.171</td>
<td>0.151</td>
<td>0.169</td>
<td>0.175</td>
<td>0.139</td>
<td>0.161</td>
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<td>Mean</td>
<td>0.164</td>
<td>0.131</td>
<td>0.170</td>
<td>0.172</td>
<td>0.136</td>
<td>0.179</td>
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<tr>
<td>SEM</td>
<td>0.017</td>
<td>0.024</td>
<td>0.015</td>
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of this observation is a possibly wider interindividual variation in SLC if determined at different phases of the menstrual cycle than if the timing of the assay were standardized in relation to a particular phase of the cycle in the same group of women.

The activity of the transporter did not correlate with oestradiol level at any stage during the experimental period (Table 2). A similar lack of relation-

ship has been reported between SLC activity and oestriol [21], and implies a lack of a modulatory effect of oestrogens on the transporter. No association was evident between progesterone level and SLC activity in the menstrual and midcycle phases, but the two variables were significantly correlated in the luteal phase (Table 2, Fig. 2a). Such a correlation does not establish a cause–effect relationship, which is probably best assessed in vitro in progesterone-containing medium that otherwise contains no potential modulator of the transport system. It, however, does not rule out an indirect interaction between the two. Indeed, the possible involvement of surrogate effector(s) would appear to be supported by the available evidence.

Activation of the renin–aldosterone system during normal menstrual cycle is well recognized. Plasma renin activity rises in the luteal phase of the menstrual cycle [22] to about two to four times that in the follicular phase [23]. The increase is due to a rise in plasma renin concentration without a change in the level of its substrate [24], and is accompanied by an increase in urinary excretion of aldosterone [25]. Aldosterone has been reported to stimulate the erythrocyte sodium/potassium pump in vitro in a dose-dependent manner [26, 27], and some [28], but not all [9, 21], investigators have observed a good correlation between the plasma level of this hormone and SLC. Similarly, plasma renin activity has been found to correlate well with SLC by most [9, 29], but not all [28], investigators. Given these observations, the idea that renin and/or aldosterone might be mediating the observed progesterone-associated increase in SLC activity in the luteal phase, though speculative, cannot be discounted outright.

Whatever the cause, it is clear that the value of SLC in females will, to some extent, depend on the timing of blood sampling during the menstrual cycle. The absence of this cyclical modulation was possibly contributory to the observed similarity in SLC in postmenopausal women and men of the same age group. The non-standardization of timing of blood sampling for SLC assay in women in their reproductive ages was also possibly contributory to the more pronounced difference in the activity of the transport system noted between this group and age-

![Graph](image)

**Fig. 2.** Correlation between plasma progesterone and SLC activity (a) and SLC V<sub>max</sub> (b) during the menstrual cycle in healthy females

<table>
<thead>
<tr>
<th>Table 2. Regression analyses of SLC activity and V&lt;sub&gt;max&lt;/sub&gt; against hormone levels during menstrual cycles in healthy subjects</th>
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matched males [7]. The same reason also possibly explains the observed lack of difference in SLC between hypertensive females and normotensive controls [2], and the report that while SLC was found to inversely correlate with age in males, no such association was evident in females [9].

**Menstrual cycle and blood pressure**

Although exogenous progestogens have been implicated in the aetiology of hypertension [31], there is a suggestion that the natural progesterone may have an anti-hypertensive action [32]. Furthermore, oestrogens are known to elevate blood pressure, albeit in pharmacological doses [33]. These observations have led many investigators to assess possible variation in blood pressure during the menstrual cycle and its relationship, if any, with the cyclical hormonal fluctuations.

A retrospective study of 207 women by Greenberg et al. [34] showed that systolic blood pressure is highest in the luteal phase (days 17–26 of the cycle). However, the same workers did not find any between-phase differences in blood pressure in a prospective study reported in the same paper. The authors ascribed the discrepant results to psychological stress resulting from the volunteers in the retrospective study being examined by previously unknown staff in an unfamiliar environment. It was suggested that the observed increase in systolic pressure in the luteal phase in the retrospective study was not due to a specific hypertensive action of progesterone but to a progesterone-mediated increase in the pressor response to stress.

The result of a later study by Kelleher et al. [35] did not support this proposition: the systolic pressure changed, and was highest at the onset of menstruation in 18 healthy subjects working in the same hospital as the investigators. More recently, other investigators [36] observed the highest systolic and diastolic pressures at the onset of menstruation in 40 healthy subjects under similar condition.

All the participants in the present study had their blood pressure taken by a known colleague and in a familiar environment (work place). Their diastolic pressures in the luteal phase (71.6±1.3 mmHg) and at the time of menstruation (70.6±1.4 mmHg) were similar, and each was higher than that in the midcycle phase (66.6±1.4 mmHg, P<0.025). Given the results of this study and those reported by others [35, 36], it is perhaps correct to say that there is consensus on the catamenial changes in blood pressure. The timing and whether or not systolic and/or diastolic pressure are involved, however, remains contentious.

In conclusion, data from the present study indicate catamenial variation in SLC, the highest activity of the transport system being in the luteal phase when progesterone level was also highest. This observation makes a case for the need to standardize the timing of blood sampling for SLC assay in women. We observed a similar pattern of change in the diastolic pressure, but systolic pressure was not altered during menstrual cycle.

**ACKNOWLEDGMENT**

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**REFERENCES**

30. Reference deleted.