An association between plasma progesterone and erythrocyte carbonic anhydrase I concentration in women

J. PACIOREK AND N. SPENCER
Department of Biochemistry, University of London, King's College, London

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Summary

1. Erythrocyte carbonic anhydrase I was measured by immunoelectrophoresis in blood samples taken at intervals during the menstrual cycle of 26 normal women. Plasma progesterone concentrations were also measured in 18 of these subjects. In every case, the erythrocyte carbonic anhydrase I concentration reached a maximum at that point in the cycle when there was maximum secretion of progesterone. Statistical analysis indicated that the observed changes in erythrocyte carbonic anhydrase I concentration are highly significant and that erythrocyte carbonic anhydrase I and plasma progesterone concentrations are significantly correlated.

2. In three pregnant subjects the erythrocyte carbonic anhydrase I concentration showed a steady increase up to term.

3. Women taking progestogens in contraceptive pills, in doses of 1 mg/day or greater, had significantly higher concentrations of erythrocyte carbonic anhydrase I compared with females receiving no medication.

4. These observations strongly suggest that there may be a causal connection between plasma progesterone concentration and erythrocyte carbonic anhydrase I concentration in women.

Key words: erythrocyte carbonic anhydrase I, oral contraceptives, menstrual cycle, plasma progesterone.

Introduction

Recently in the course of another study we measured carbonic anhydrase (EC 4.2.1.1) isoenzymes I and II in human erythrocytes: in males aged 19–22 years values were obtained of 12.9–17.8 mg/g of haemoglobin for carbonic anhydrase I and 1.6–2.7 mg/g for carbonic anhydrase II, and these are similar to previously reported values (Tashian, 1977); in a corresponding group of young females, however, the concentration of erythrocyte carbonic anhydrase I, but not of carbonic anhydrase II, showed considerable variation. This variation appeared to be associated with the use of oral contraceptives and confirms earlier observations that the amounts of carbonic anhydrase I found in erythrocytes may be affected by the use of contraceptive pills (Auton, Barragry, Carter, Morris & Cohen, 1976).

To assess the significance of possible effects of orally administered steroids on the concentration of erythrocyte carbonic anhydrase I, it was necessary to measure carbonic anhydrase I during the menstrual cycle to determine the extent of normal fluctuations in the concentration of this enzyme. This report presents the results of such a survey in 26 women. Where possible, plasma progesterone concentrations were also measured to provide monitoring of the cycle.

Materials and methods

Blood was obtained from student nurses at Crawley Hospital (Sussex), who volunteered to take part in these experiments. A group of 26 nurses who...
were not taking contraceptive pills each provided blood samples every 4 days throughout the course of one complete menstrual cycle; from a further group of 88 nurses who were not taking contraceptive pills single samples were obtained. Single samples from 49 nurses who were using various types of contraceptive pill were also obtained. Portions of blood taken for routine testing were obtained from three pregnant subjects attending the same hospital.

Blood, collected in lithium heparin bottles, was centrifuged at 600 g for 10 min and the plasma removed and stored at −20°C until analysed. After removal of the buffy coat layer the erythrocytes were washed three times by resuspension and centrifugation as above in sodium chloride solution (154 mmol/l: saline). Finally, packed erythrocytes were lysed by freezing and thawing and stored at −20°C until analysed. The immunoelectrophoresis technique described by Nørgaard-Pedersen (1973) was used to measure carbonic anhydrase I in erythrocyte lysates. One minor modification was adopted when it was found necessary to extend treatment with potassium cyanate to 16 h in order to carbamylate the enzyme fully; immediately after addition of potassium cyanate to lysates, portions (100 μl) were removed for haemoglobin assay in Drabkin’s solution (Crosby, Munn & Furth, 1954). Dilutions of standard solutions of carbonic anhydrase I or carbonic anhydrase II where appropriate were run alongside unknown samples on each electrophoresis plate; repeated estimations at several dilutions gave a coefficient of variation that did not exceed 2-6%.

Pure samples of carbonic anhydrase I and carbonic anhydrase II were prepared according to the method of Armstrong, Myers, Verpoorte & Edsall (1966) and both enzymes were found to be homogeneous on electrophoresis in polyacrylamide gels (Laemmli, 1970). Specific antisera to carbonic anhydrase I and carbonic anhydrase II were raised in New Zealand white rabbits by following the procedures described by Harboe & Ingild (1973). Antiserum to carbonic anhydrase I was also obtained from Hoenchst (U.K.) Ltd, Hounslow, Middlesex, U.K.

Plasma progesterone concentration was measured in the method of Collins & Hennam (1976). Coefficient of variation was 7% for intra-assay and 14% for inter-assay. Limit of detection at best precision was 0-3 nmol/l.

Antisera to progesterone-11α succinyl bovine thyroglobulin raised in rabbits were supplied by the World Health Organization and [1,2,6,7-3H]-pregn-1,4,6-triene-3,20-dione was obtained from The Radiochemical Centre, Amersham.

Results

Since the original objective of this work was to measure the extent of any variation in erythrocyte carbonic anhydrase I concentration during the normal menstrual cycle, accurate monitoring of the cycle was not required. For present purposes samples taken between the onset of menstruation and 14 days into the cycle are designated pre-ovulation, and those taken after 14 days and before the next menstruation are designated post-ovulation. Carbonic anhydrase I was measured in three post- and three pre-ovulation samples taken from each of 26 subjects: the relevant data have been deposited as Clinical Science Table no. 79/6 with the Librarian, the Royal Society of Medicine, 1 Wimpole Street, London W1M 8AE, from whom copies can be obtained. The samples from 18 of these subjects were also analysed for plasma progesterone and these data have been similarly deposited as Clinical Science Table no. 79/7. For those subjects in whom both erythrocyte carbonic anhydrase I and progesterone concentrations were measured correlation coefficients were calculated: with one exception (r = 0.86) all the coefficients exceeded 0.9 and the value of r was significant in 14 subjects and probably significant in the remaining four subjects. Without exception the highest concentration of erythrocyte carbonic anhydrase I coincided with the highest concentration of plasma progesterone.

Post-ovulation values for erythrocyte carbonic anhydrase I were higher than pre-ovulation values in all subjects; when the difference between means of pre- and post-ovulation concentrations are expressed as percentages of the mean pre-ovulation value for each subject, the differences range from 3-3 to 10-7% (mean value 7-2%). The difference is more marked when mean pre-ovulation concentrations are compared with those measured in samples corresponding to the ‘progesterone peak’ (Fig. 1). A comparison of paired means of pre- and post-ovulation concentrations for carbonic anhydrase I gave t = 19-3 (P < 0-001).

Further evidence for an association between plasma progesterone and erythrocyte carbonic anhydrase I concentrations is provided by the data summarized in Fig. 2, which show that the concentration of erythrocyte carbonic anhydrase I increases steadily up to term in pregnancy.
Progesterone and erythrocyte carbonic anhydrase I

Data for erythrocyte carbonic anhydrase I concentration in women taking three different types of contraceptive pill are presented in Table 1. The concentration of erythrocyte carbonic anhydrase I in women taking either of the pills containing 1 mg or more of progestogen is significantly higher than that measured in women receiving no medication; further, the carbonic anhydrase I concentration was significantly higher in women taking more than 1 mg of progestogen/day than in women taking 0.5–1.0 mg/day.

Discussion

The combined data presented here suggest that there may be a causal connection between increases in plasma progesterone concentration and increases in the concentration of erythrocyte carbonic anhydrase I. The results of analyses on samples taken at intervals during the menstrual cycle provide the strongest evidence for such a connection. There is a clear-cut and significant rise in erythrocyte carbonic anhydrase I concentration that exactly parallels changes in plasma progesterone concentration and reaches a maximum at a point in the cycle corresponding to the peak of progesterone secretion.

It is well established that plasma progesterone concentration increases regularly after the first few weeks of pregnancy (Catt, 1971), and it is clear from the present data (Fig. 2) that erythrocyte carbonic anhydrase I concentration also increases steadily; similar changes in erythrocyte carbonic anhydrase I concentration during pregnancy have been noted by others (Schenker, Ben-Yoseph & Shapka, 1972; Auton et al., 1976).

The data relating erythrocyte carbonic anhydrase I concentration to the progesterone content of different types of contraceptive pill are more difficult to interpret because the pills also contain oestrogens. However, it is probably valid to compare the erythrocyte carbonic anhydrase I concen-

![Graph](image1)

**Fig. 1.** Correlation between plasma progesterone and erythrocyte carbonic anhydrase I concentrations. •, Mean value for three pre-ovulation samples; ▲, single value corresponding to peak of progesterone secretion. Each symbol represents a single individual. Hb, Haemoglobin.

![Graph](image2)

**Fig. 2.** Erythrocyte carbonic anhydrase I concentration at various times during pregnancy. Each symbol represents a single individual. Hb, Haemoglobin.

<table>
<thead>
<tr>
<th>Type</th>
<th>Dose (µg/day)</th>
<th>Erythrocyte carbonic anhydrase I* (mg/g of haemoglobin)</th>
<th>No. of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oestrogen</td>
<td>Progesterone</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>30</td>
<td>150–500</td>
<td>10.8 (1.7)</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>500–1000</td>
<td>16.3 (1.9)</td>
</tr>
<tr>
<td>3</td>
<td>50–1000</td>
<td>2500–4000</td>
<td>23.0 (1.3)</td>
</tr>
</tbody>
</table>

* Mean value with SD in parentheses. Normal range for 114 females of a similar age group not taking medication: 12.3–17.6, mean 14.4 (1.5) mg/g of haemoglobin.
tration in women taking the type 2 pill with that in women taking the type 3 pill (Table 1), as both pills contain 50 μg of oestrogen; such a comparison reveals a significantly higher concentration of erythrocyte carbonic anhydrase I in women receiving the higher dose of progesterone. A direct relationship between progesterone dosage and carbonic anhydrase activity in human endometrium has previously been reported (Nicholls & Board, 1967).

The possibility that changes in blood progesterone concentration might affect carbonic anhydrase has been recognized since 1955, when increased activity of the enzyme was observed in rabbit endometrium in response to a rise in blood progesterone (Lutwak-Mann, 1955). More recently Falk & Hodgen (1971) demonstrated increased carbonic anhydrase activity in the human endometrium during the luteal phase of the menstrual cycle.

One possible explanation of the present data is that progesterone might stimulate synthesis of carbonic anhydrase I only in circulating erythrocytes capable of some protein synthesis, i.e. reticulocytes; these represent about 1% of circulating erythrocytes and direct evidence for the synthesis of carbonic anhydrase I in such cells has been obtained through the use of radioactively labelled amino acids (Myers, Brewer & Tashian, 1969; Edwards, 1970). A difficulty with this suggestion concerns the transient nature of the observed increase in the amount of erythrocyte carbonic anhydrase I during the menstrual cycle; the possibility that progesterone might stimulate the synthesis of a form of the enzyme that is immuno-logically identical but which has a considerably shorter half-life should not be discounted.

Acknowledgments

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References


