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Regional differences in energy charge of the pregnant human uterus regardless of functional status in comparison with the non-pregnant uterus

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The energy status of the cell is mainly dependent on adenine nucleotides and can be expressed as energy charge (EC). EC is known to be kept at narrow limits near 0.90 under normal conditions in most cells. We recently reported remarkably low EC values in the human uterus under apparent steady-state conditions. The present paper is an extension of previous work. It shows that EC varies in different regions of the uterus in that the isthmic part in pregnant women displays a higher EC than the fundus of the uterus. There were no intergroup differences between non-pregnant and term pregnant women, nor between those who were in active normal labour, dysfunctional labour or those who were not in labour at all. On the other hand, EC in uterine muscle of post-menopausal women showed a significantly lower EC value. Human uterus seems to manage its metabolic requirements under different functional conditions in spite of low ATP and EC values. This suggests that ATP occurs in sufficient amounts to pertinent enzyme reactions, especially ATPases, which means K_m values adapted for this unusually low ATP concentration.

Introduction

The energy status of the cell is dependent mainly on adenine nucleotides, i.e., ATP, its interconvertible analogues and creatine phosphate (CP). One index of energy status is the energy charge (EC) as proposed by Atkinson [1], which is proportional to the mole fraction of ATP plus half the mole fraction of ADP, given that ATP contains two anhydride bonds, whereas ADP contains one. This charge is maintained within rather narrow limits under normal conditions in most cells. ATP-utilizing pathways are stimulated by high EC, whereas ATP-producing pathways are stimulated by low EC.

Recently we described an unexpectedly low EC close to 0.60 in the steady state for uterine smooth muscle concomitantly with low values for total adenine nucleotides and CP. Since the pregnant uterus may be considered to be functionally consisting of different parts and to be heterogeneous with regard to hormonal influences, it was of interest to investigate EC in different parts of the human uterus. Moreover, we wanted to investigate whether uterine contractility pattern or absence of hormonal influence, i.e., the post-menopausal state, might affect EC. The present paper describes EC in different parts of the pregnant human uterus as well as in uterus of different functional status.

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Materials and Methods

Patients

The patient material comprised 21 pregnant women (age 21–38 years), 14 non-pregnant women (age 22–39 years) and 8 post-menopausal women (age 56–77 years) (Table I). All pregnant women were subjected to Caesarean section. Seven were operated upon before onset of labour, because of breech presentation or known pelvic contraction. Fourteen others were in active labour. Ten of them had seemingly normal contractions but were operated upon mainly on foetal indications such as foetal distress or malpresentation. Four had abnormal contractions (primary dystocia) refractory to oxytocin therapy necessitating operative delivery. 13 non-pregnant women were undergoing laparotomy for sterilization. The third and last group comprised 8 post-menopausal women operated upon because of ovarian cysts or early endometric cancer. In

TABLE I

Patient data (41 women)

Patients	*n*	Mean age (y)	Births (mean)	Indications
Pregnant				
Caesarean section				
elective	7	30	2.1 [a]	breech presentation, pelvic contraction
emergency	4	28	1.5 [a]	primary dystocia
emergency	10	29	1.1 [a]	foetal distress, malpresentation
Non-pregnant				
(pre-menopausal)	14	40	1.8	contraceptive sterilisation bleeding disorders
Post-menopausal	8	69	2.4	ovarian cysts

[a] Including the present delivery by Caesarean section.

all cases the uterus appeared normal at the biopsy site. All biopsies were taken after informed consent and the study was approved by the Ethical Committee of the University of Uppsala.

Biopsy technique

Uterine biopsies were taken from the uterine fundus in all three groups and from the isthmic part from only the pregnant group. The isthmus biopsy was obtained directly after delivery but before uterine contraction, while the fundus biopsy was taken after suturing of the hysterotomy and uterine contraction. From all patients a biopsy was also taken from the rectus abdominis muscle. The biopsies intended for biochemical analyses were handled according to a standard routine. Immediately after the biopsy had been taken, it was dipped into ice-cold isotonic saline and instantly transferred into liquid nitrogen. Therewith, the time period from excision until freezing in liquid nitrogen was less than 10 s.

Biochemical methods

After lyophilization and acid extraction the specimens were analyzed for adenine nucleotides, CP and lactate contents. The adenine nucleotides were determined by high performance liquid chromatography (HPLC) according to previously described methods [2]. The equation of Atkinson was adopted for calculation of EC [1]. Creatine phosphate and lactate were measured by enzymatic methods [3]. Collagen content was calculated from hydroxyproline measurements by a colorimetric method after appropriate extraction procedures [4].

Statistical methods

One-way analysis of variance (ANOVA) with least

TABLE II

Energy charge (EC), total nucleotide content (TNC) adenylates, creatine phosphate (CP) and lactate in fundus biopsies and striated muscle (rectus abdominis)

Number of patients (n), mean values in μmol/g d.wt. (except for EC) and 95% least significant difference confidence intervals for means (c.i.) and *P* value are given. The *P* values refer to comparison with non-pregnant, pre-menopausal myometrium.

	Non-pregnant ($n=14$)		Pregnant ($n=20$)			Post-menopausal ($n=8$)			Rectus abdominis ($n=33$)		
	mean	(c.i.)	mean	(c.i.)	*P*	mean	(c.i.)	*P*	mean	(c.i.)	*P*
EC	0.60	(0.56– 0.65)	0.52	(0.48– 0.56)	n.s.	0.32	(0.26– 0.38)	0.01	0.90	(0.87– 0.93)	< 0.001
TNC	5.81	(4.42– 7.19)	5.35	(4.19– 6.51)	n.s.	3.42	(1.59– 5.25)	0.05	13.39	(12.50–14.27)	0.001
ATP	2.79	(1.71– 3.87)	1.95	(1.05– 2.84)	n.s.	0.50	(−0.92– 1.92)	0.01	11.01	(10.32–11.70)	0.01
ADP	1.97	(1.58– 2.36)	1.98	(1.65– 2.31)	n.s.	1.12	(0.61– 1.64)	0.05	2.19	(1.94– 2.44)	n.s.
AMP	1.05	(0.85– 1.25)	1.40	(1.23– 1.57)	n.s.	1.76	(1.53– 2.06)	0.05	0.23	(0.10– 0.36)	< 0.001
CP	7.41	(5.18– 9.64)	9.58	(7.71–11.45)	n.s.	14.68	(11.72–17.63)	0.05	17.45	(16.02–18.88)	0.001
Lactate	27.83	(23.68–31.98)	40.33	(37.12–43.55)	0.01	32.50	(27.42–37.58)	n.s.	19.11	(16.57–21.65)	0.05

significant difference (LSD) as ad hoc test was used for comparison of the mean values. A $P < 0.05$ was regarded as statistically significant. A linear regression and Pearson's coefficient was used for correlation studies.

Results

Non-pregnant, pregnant and postmenopausal uteri showed low EC compared with rectus muscle (Table II). The EC and ATP contents tended to be slightly lower in pregnant uterus compared with non-pregnant uterus, although the differences were not statistically significant. The lactate content was, however, significantly higher in pregnant myometrium compared with non-pregnant myometrium. The levels of ADP, AMP and CP did not differ between these two patient groups (Table II). In postmenopausal patients the contents of all adenine nucleotides were significantly lower than in non-pregnant as well as pregnant patients. The most striking difference was a very low ATP content despite a rather high uterine content of CP in these patients. This finding combined with a moderately elevated content of AMP was manifested by an extremely low EC of only 0.32 (Table II).

Biopsies from fundus uteri displayed significantly lower ATP and EC values than biopsies from the isthmic region in pregnant women. Regarding AMP an inverse relationship was observed. Similarly, the lactate content in isthmic region was lower than in the fundus region (Table III). The pregnant women undergoing Caesarean section were subdivided in three groups with regard to the myometric functional status. Those operated upon before onset of labour displayed EC values in their fundus and isthmus region that were 0.56 and 0.76, respectively, i.e., reflecting a higher value of the isthmic part which was consistent for also the other two groups, viz. those having primary dystocia and normal labour (Table IV). The same pattern was conspicuous for ATP and the expected reciprocity of AMP values was noted (Table IV).

In order to exclude a dilution effect due to different contents of, for example, collagen when expressing phosphocompounds on a dry weight basis, analysis of collagen was performed. The collagen content of isth-

TABLE III

Energy charge (EC), total nucleotide content (TNC), adenylates, creatine phosphate (CP) and lactate in fundus biopsies from pregnant patients compared with isthmus biopsies from the same individuals

Number of patients (n), mean values in μmol/g d.wt. (except for EC), 95% least significant difference confidence intervals for means (c.i.) and P value (P) are given.

	Fundus ($n = 20$)		Isthmus ($n = 21$)		
	mean	(c.i.)	mean	(c.i.)	P
EC	0.52	(0.48– 0.56)	0.71	(0.67– 0.75)	< 0.001
TNC	5.35	(4.19– 6.51)	6.45	(5.32– 7.58)	n.s.
ATP	1.95	(1.05– 2.85)	3.87	(2.99– 4.74)	0.01
ADP	1.98	(1.65– 2.31)	2.03	(1.71– 2.35)	n.s.
AMP	1.40	(1.23– 1.57)	0.52	(0.35– 0.68)	< 0.001
CP	9.58	(7.71–11.45)	9.10	(7.27–10.92)	n.s.
Lactate	40.33	(37.12–43.55)	33.83	(30.70–36.97)	0.05

TABLE IV

Energy charge (EC), total nucleotide content (TNC), adenylates, creatine phosphate (CP) and lactate in uterine fundus (F) and isthmus (I) biopsies

Before labour denotes patients subjected to elective surgery before onset of labour. *Dystocia* denotes patients in labour operated upon due to intractable prinary dystocia, and *normal labour* denotes patients with seemingly normal contractions but operated upon due to signs of foetal distress or malpresentation. Number of patients (n), mean values in μmol/g d.wt. (except for EC) and 95% least significant difference confidence intervals for means (c.i.) are given. None of the differences between functional groups was statistically significant.

		Before labour ($n = 7$)		Dystocia ($n = 4$)		Normal labour ($n = 10$)	
		mean	(c.i.)	mean	(c.i.)	mean	(c.i.)
EC	F	0.56	(0.49– 0.63)	0.51	(0.41– 0.61)	0.49	(0.43– 0.55)
	I	0.77	(0.69– 0.83)	0.75	(0.65– 0.85)	0.66	(0.60– 0.72)
TNC	F	6.52	(4.79– 8.25)	2.65	(0.36– 4.94)	5.25	(3.81– 6.70)
	I	7.65	(5.92– 9.38)	5.65	(3.36– 7.34)	5.92	(4.48– 7.37)
ATP	F	2.65	(1.40– 3.90)	0.95	(−0.71– 2.61)	1.70	(0.65– 2.75)
	I	5.02	(3.78– 6.28)	3.34	(1.68– 5.00)	3.26	(2.21– 4.31)
ADP	F	2.39	(1.90– 2.41)	1.02	(0.37– 2.89)	1.99	(1.57– 2.41)
	I	2.13	(1.64– 2.63)	1.86	(1.20– 2.52)	2.02	(1.60–2.43)
AMP	F	1.43	(1.13– 1.74)	0.67	(0.26– 1.08)	1.56	(1.30– 1.82)
	I	0.38	(0.07– 0.69)	0.44	(0.04– 0.86)	0.64	(0.38– 0.90)
CP	F	10.08	(7.00–13.16)	7.68	(3.61–11.76)	9.88	(7.31–12.46)
	I	9.61	(6.54–12.70)	8.35	(4.28–12.43)	9.03	(6.45–11.61)
Lactate	F	38.08	(32.75–43.42)	34.70	(27.64–41.76)	43.80	(39.33–48.26)
	I	32.25	(26.91–37.58)	33.33	(26.27–40.39)	35.14	(30.68–39.61)

TABLE V

Collagen content in uterine isthmus and fundus biopsies and rectus abdominis muscle

Mean values in mg/g w.wt., 95% least significant difference confidence intervals for means (c.i.), number of patients (n) and P values are given. The P values refer to comparison with non-pregnant (fundal) myometrium.

	Non-pregnant				Pregnant				Post-menopausal			
	mean	(c.i.)	n	P	mean	(c.i.)	n	P	mean	(c.i.)	n	P
Isthmus	–	–			7.47	(6.55–8.39)	20	0.05	–	–		
Fundus	10.38	(8.54–12.22)	5	–	8.65	(7.62–9.68)	16	n.s.	10.69	(9.24–12.15)	8	n.s.
Rectus	6.47	(3.52–9.41)	2	n.s.	5.95	(3.52–9.42)	11	n.s.	5.33	(3.47– 7.20)	5	0.05

mus uteri was slightly lower than that of fundus uteri in pregnant women (Table V). There were no differences in this regard in fundus biopsies between non-pregnant, pregnant and post-menopausal uterine tissue. The rectus muscle displayed consistently lower values, although reaching a level of statistical significance only in the post-menopausal group (Table V). No correlation was found between collagen contents and ATP, total nucleotide content or EC in uterine tissue of these patients regardless of functional status.

Discussion

EC was strikingly low in the human uterine tissue. This confirms our earlier published data [5]. In the present study the low EC was demonstrated irrespective of uterine functional status. This means, that there were no intergroup differences regarding non-pregnant and pregnant women, those in active labour and those with primary dystocia refractory to oxytocin therapy and those having seemingly normal contractions. This again demonstrates that EC is maintained within narrow limits also in human uterine tissue regardless of metabolic state and work load. This is also congruent with other tissues, although the EC values of uterus are significantly lower than in all other investigated tissues. Seemingly, there is no simple relationship between energy requirement and the EC value. Energy charge in smooth muscle has been little investigated (cf. Ref. 6).

Uterus from post-menopausal women did not entirely fit into this pattern in that its EC was significantly lower. The reason for this is not fully understood. ATP values parallelled the EC in a conspicuous way, which is not unexpected, since ATP is one of the determinants of the EC value. Moreover, the CP value displayed an inverse relationship in that the uterine CP in post-menopausal women was significantly higher than in fertile women. The polarity between ATP and CP in post-menopausal uterine tissue indicates an influence on the creatine kinase reaction in that the reaction is in favour of CP formation. The nature of this influence has not been investigated so far. The equilibrium of this reaction is dependent on the ambient tissue pH as well as ADP and total creatine [7]. Unfortunately, we have no information on the tisue pH but the lactate levels do not suggest any metabolic acidosis over and above what can be assumed to exist in a pregnant and non-pregnant tissue with clearly discordant ATP/CP relationships.

The low ATP value calculated on a mass unit basis is not simply a dilution effect due to e.g., abundance of connective tissue. Several lines of evidence contradict such an explanation. Since the biopsy technique concerning uterus and rectus muscle was exactly the same the metabolic data can be compared. The high CP level of the rectus muscle is in accordance with earlier findings in this laboratory reflecting representativity of the specimens. This indicates that the actual CP-value of uterine muscle is lower. The higher lactate level of such a representative uterine biopsy indicates a higher glycolytic flux in this tissue. As it may be argued that biopsy site for the EC analysis was not relevant for the uterus as such, two predetermined loci were selected well apart (i.e., isthmus and fundus regions). Still low EC values were registered in both loci, although the pattern was discerned so that the fundus uteri displayed significantly lower ATP and EC values than the isthmus region while the collagen content was about the same in these two loci. This again contradicts a simple relationship between EC and ATP on one hand and the requirements of chemical energy on the other hand, as the fundus region takes active part in labour and isthmus only relaxes in this situation. Although we cannot offer any simple mechanistic explanation to date for these topographical differences, it can be noted that cervix uteri with a passive role has a higher EC.

We conclude that human uterus manages its metabolic requirements under different functional conditions in a steady-state fashion in spite of low ATP concentration and low EC values. This implies that the K_m values of ATP-utilizing enzymes are adapted to a low ATP concentration. A corollary is that those ATPases might exhibit unique kinetic properties.

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