DEPENDENCE OF THE MOTOR FUNCTION OF THE RABBIT UTERUS ON ACTIVITY OF THE ENZYMES OF GLUCOSE METABOLISM

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The motor function of the uterus of intact and pregnant rabbits was investigated in vitro during inhibition of the key enzymes of the pentose phosphate cycle (glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase) and of the Embden–Meierhof–Krebs cycle (glyceraldehyde-3-phosphate dehydrogenase, enolase, and succinate dehydrogenase). Under these conditions regular changes took place in the contractile function of the uterine cornua. During pregnancy activation of the enzymes responsible for direct oxidation of glucose in the pentose phosphate cycle was observed in the smooth-muscle cells of the myometrium, and this could be a factor maintaining pregnancy.

KEY WORDS: uterus; pregnancy; glucose metabolism.

Despite numerous investigations of the functional and structural features of the uterus [1, 2, 5-8, 11-13, 18], the concrete mechanisms whereby metabolism in the myometrial cell is coupled with the motor function of the uterus have not yet been discussed. The role of the pentose phosphate pathway of glucose conversion for the repolarization of the cell membranes and passage of the cell into a state of rest has been established in principle during recent years [14, 17], and differences in the contractile function of smooth-muscle organs (intestine, blood vessels) depending on the predominance of one particular pathway of glucose metabolism have been discovered [15, 16].

It was therefore decided to compare the character of the contractile function of the rabbit uterus with the activity of certain enzymes participating in glucose metabolism in the myometrial smooth-muscle cell.

EXPERIMENTAL METHOD

Segments of the uterine cornu of intact and pregnant rabbits were kept in Kravkov's solution (38°C) through which oxygen was passed continuously at a constant rate of flow.

Enzymes of the pentose phosphate cycle – glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD) – were inhibited with copper sulfate (0.33-0.66 mg%). The Embden–Meierhof pathway was blocked by monoiodoacetate (12.5-25 mg%), which inhibits glyceraldehyde-3-phosphate dehydrogenase, and sodium fluoride (4-8 mg%), which inhibits inolase. Succinate dehydrogenase (SD) in the Krebs cycle was blocked by malonate (12.5-25 mg%).

G6PD and 6PGD activity in the soluble fraction of the uterine tissue from intact and pregnant rabbits was determined by the well-known method of Glock and McLean and expressed in optical density units/100 mg protein/min. The activity and localization of these enzymes in the uterus was also investigated histochemically [20]. Ribose-5-phosphate isomerase (R5PI) [9] and transketolase (TK) [10] activity was ex-

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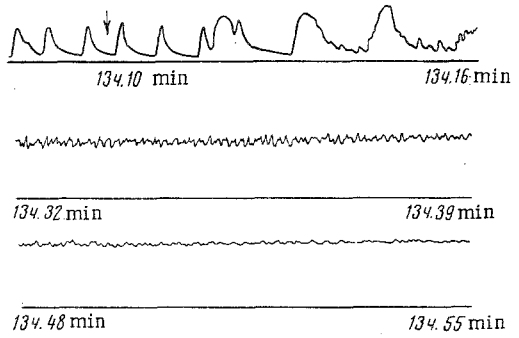

Fig. 1

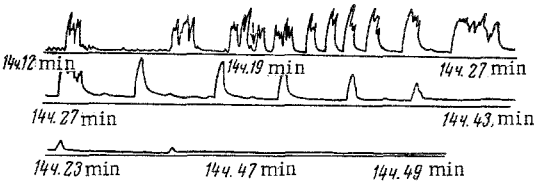

Fig. 2

Fig. 1. Effect of copper sulfate on contractions of isolated rabbit uterine cornu: 20th day of pregnancy. Arrow marks addition of copper sulfate in a final concentration of 0.66 mg%.

Fig. 2. Inhibition of contractions of isolated rabbit uterine cornu by monoiodoacetate: 20th day of pregnancy. Arrow marks addition of monoiodoacetate in concentration of 12.5 mg%.

TABLE 1. Enzyme Activity in Smooth-Muscle Cells of Rabbit Myometrium during Pregnancy (M ± m)

Group of animals	G6PD (in optical density units/100 mg protein/min)	6PGD (in optical density units/100 mg protein/min)	R5PI (in optical density units/mg protein/15 min)	TK (in optical density units/mg protein/60 min)	PFK (in units/mg protein/min)	Aldolase (in units/mg protein/min)
1	0,368 ± 0,06	0,158 ± 0,03	0,314 ± 0,03	0,199 ± 0,043	1,51 ± 0,18	2,95 ± 0,46
2	0,605 ± 0,06	0,56 ± 0,07	0,895 ± 0,109	0,238 ± 0,02	2,49 ± 0,21	3,97 ± 0,4
	$P < 0,01$	$P < 0,01$	$P < 0,01$	$P > 0,1$	$P < 0,01$	$P > 0,1$
3	0,410 ± 0,43	0,360 ± 0,04	0,390 ± 0,03	0,218 ± 0,03	3,207 ± 0,26	4,5 ± 0,74
	$P > 0,1$	$P < 0,01$	$0,1 > P > 0,05$	$P > 0,1$	$P < 0,01$	$P > 0,1$
4	0,530 ± 0,1	0,471 ± 0,1	0,424 ± 0,05	0,193 ± 0,07	3,32 ± 0,36	3,84 ± 0,22
	$P > 0,1$	$P < 0,01$	$0,1 > P > 0,05$	$P > 0,1$	$P < 0,01$	$0,1 > P > 0,05$

pressed in optical density units/mg protein/15 min for R5PI and /60 min for TK. Phosphofructokinase (PFK) [3] and aldolase [4] activity was expressed in units/mg protein/min incubation. SD activity was determined histochemically [19].

The animals as a whole were divided into four groups: 1) intact rabbits; 2) rabbits on the 9th-10th day of pregnancy; 3) on the 19th-20th day of pregnancy; 4) on the 29th-30th day of pregnancy. Tests were carried out on 40 animals.

EXPERIMENTAL RESULTS AND DISCUSSION

Blocking the dehydrogenases of the pentose phosphate cycle by copper sulfate significantly changed the motor function of the uterus in both intact and pregnant rabbits. As a rule the frequency and amplitude of the contractions were increased and after 8-10 min the tone also was increased. In some cases tonic contraction became stable 40-60 min after the addition of copper sulfate (Fig. 1).

Addition of monoiodoacetate significantly reduced the amplitude and frequency of the uterine contractions or in some cases completely suppressed them (Fig. 2). Addition of sodium fluoride caused definite stimulation of uterine contractions: an increase in the tone and amplitude and frequency of the contractions. The addition of malonate led to a progressive decrease in amplitude or even complete suppression of uterine contractions.

Data showing the activity of various enzymes of glucose metabolism in the smooth-muscle cells of the myometrium are given in Table 1. To judge from these data, activation of several enzymes of the pentose phosphate cycle (G6PD), 6PGD, and R5PI) occurred during pregnancy, especially on its 9th-10th day.

On the 29th-30th day of pregnancy PFK activity was doubled. By this time a tendency toward an increase in aldolase activity also was observed.

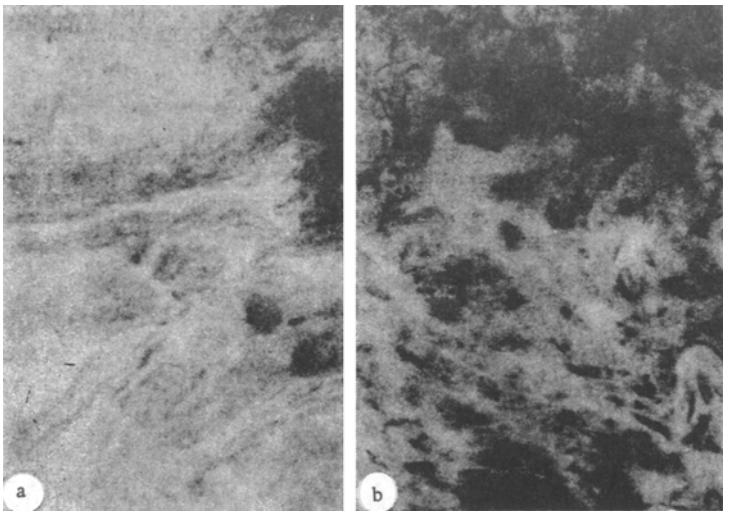

Fig. 3. G6PD activity in outer longitudinal layer of uterus of intact rabbit (a), and of rabbit on 30th day of pregnancy (b). Histochemical reaction after Scarpelli, Hess, and Pearse, 63×.

The histochemical tests showed that G6PD and 6PGD activity increased during pregnancy both in the muscular layers and in the mucous membrane of the myometrium (Fig. 3). SD activity also increased during pregnancy and was highest (++++) in the cytoplasm of the smooth-muscle cells of the middle layer of the myometrium and in the cytoplasm of the epithelial layer of the mucous membrane.

The results demonstrate that blocking the oxidative branch of the pentose phosphate cycle, and also of the Embden–Meierhof–Krebs cycle, is accompanied by regular changes in the contractile function of the rabbit uterus. This suggests that the smooth-muscle cells of the rabbit myometrium belong to type B (balanced) in Laborit's classification [15], a type characterized by the presence of enzymes for both pathways of glucose conversion. This conclusion is confirmed by the results of determination of the activity of several of these enzymes in the smooth-muscle cells of the myometrium.

During pregnancy the enzymes responsible for the direct oxidation of glucose in the pentose phosphate cycle are activated; this is very important for the repolarization of the cell membranes, the accumulation of potassium inside the cell, and its relaxation and transition into a state of rest [15].

Activation of the pentose phosphate cycle during pregnancy can be regarded as an adaptive response of the smooth-muscle cell rendering the myometrium relatively inert toward the action of humoral and nervous stimuli, and thus favoring the continuation of pregnancy.

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