

INFLUENCE OF OESTRADIOL AND PROGESTERONE INJECTIONS ON THE ACID-SOLUBLE PHOSPHATE FRACTIONS OF THE RAT UTERUS*

PIERRE VOLFIN, HUBERT CLAUSER AND DANIELÈ GAUTHERON

Laboratoire de Chimie biologique de la Faculté des Sciences, Paris (France)

The influence of physiological conditions and ovarian hormones on the acid-soluble phosphate fractions of the uterus has been studied by several authors within the last few years. WALAAS AND WALAAS¹ found extremely low values for ATP and phosphocreatine and no significant effect of oestradiol or oestradiol plus progesterone on these values in the uterus of the ovariectomized rat. BORELL^{2,3} found small ATP values in the uterus of ovariectomized rabbits. Oestradiol and progesterone had no effect on the percentage of high-energy phosphates as compared with the total acid-soluble phosphorus, but they induced striking changes in the turnover of the various phosphate fractions. MENKES AND CSAPO⁴ cast some doubt on these very low values of high-energy phosphates since they were obtained on the isolated myometrium and thus may be due to a loss occurring during the handling of the muscle. They studied the variations of the "true" inorganic and high-energy phosphates throughout the sexual cycle of the rabbit uterus: noticeable quantities of phosphocreatine and ATP were found by these authors in the mature rabbit. No phosphocreatine was found in the immature rabbit uterus. However, no reinvestigation of the various phosphate fractions of the uterus with respect to the influence of ovarian hormones has been undertaken.

The present work** shows that the rat uterus contains very noticeable quantities of phosphocreatine- and ATP-like high-energy phosphates and that there are considerable variations of these esters under the influence of ovarian hormones.

METHODS

Immature, adult or spayed Wistar rats have been used. Spayed rats were used 4 weeks exactly after ovariectomy. Oestradiol and progesterone were injected subcutaneously in neutralized peanut oil, on two consecutive days. In the case of rats injected with progesterone and oestradiol, both hormones were injected simultaneously. In all cases the rats were killed by decapitation 36 hours after the last injection, the uterus excised as quickly as possible (about 1 minute) and frozen in liquid nitrogen. Homogenization, centrifugation and phosphorus determinations were carried out as previously described⁵: "true" inorganic phosphorus, according to LOWRY AND LOPEZ⁶; 0 min P ("true" inorganic phosphorus plus easily hydrolysable fractions of the phosphocreatine type), 7 min P (energy-rich phosphate of the ATP type, hydrolysed by a 7 minutes treatment with *N* HCl at 100° in a sealed tube), 180 min P (phosphate hydrolysed by a 180 minutes treatment with *N* HCl at 100° in a sealed tube) and total acid-soluble P (after digestion

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TABLE I

Group	Conditions (number of rats)	Number of experiments	Average weight (g)	Average uterus fresh weight (mg)	"True" inorganic P
1	Immature untreated animals (32)	3	41	18.7	20.1 \pm 0.9
2	Immature oestradiol-treated animals (oestradiol benzoate $2 \times 10 \mu\text{g}$) (20)	4	44	95.5	11.7 \pm 0.4
3	Immature oestradiol and progesterone-treated animals (oestradiol benzoate $2 \times 10 \mu\text{g}$) (progesterone $2 \times 100 \mu\text{g}$) (19)	4	50	102.3	6.9 \pm 0.3
4	Adult untreated animals (7)	6	206	291.4	18.6 \pm 1.0
5	Adult ovariectomized animals (16)	9	222	83.8	13.4 \pm 0.5

All phosphorus values are expressed in μg P per 100 mg uterus fresh weight \pm standard error of the mean.

with HClO_4) were determined according to ZELLER⁷ and FISKE AND SUBBAROW⁸. Standards and internal standards, in order to check the absence of colour inhibitors, were routinely performed with the real assays.

RESULTS

The results are summarized in Table I. An excellent agreement between ZELLER's and FISKE AND SUBBAROW's methods was obtained. Hence in Groups 1 and 5 it was found unnecessary to use both methods in parallel.

It is obvious that oestradiol injections on the immature rat (Groups 1 and 2) provoke a striking increase of the phosphocreatine (+170%) and ATP (+100%) contents and a decrease in "true" inorganic phosphates (−42%). When progesterone is injected in addition to oestradiol (Group 3), the ATP level remains the same as in the case of the oestradiol-injected animals, but the phosphocreatine-like fraction reaches a very high value (+335% as compared with the uninjected controls), at the expense of true inorganic phosphate (−66% as compared with the uninjected controls). All these changes are highly significant: $P < 0.01$.

Groups 4 and 5 show the effect of ovariectomy on adult rats. Surprisingly enough the high-energy phosphate level rises after ovariectomy and this rise is significant as far as phosphocreatine is concerned: +90%: $P < 0.05$.

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0 min P	Phosphocreatine-like esters	7 min P	180 min P	Total acid-soluble P
3.9 ± 0.9	3.2 ± 0.4	7.3 ± 0.3	15.9	93.5 ± 4.1
0.3 ± 1.2 od 1	8.7 ± 1.6	14.5 ± 1.0	25.4 ± 0.9	68.2 ± 4.5
0.5 ± 1.7 od 2	8.9 ± 1.7	14.4 ± 1.0	—	71.0 ± 5.9
0.8 ± 1.1	9.4 ± 1.7	14.3 ± 1.4	—	68.7 ± 1.7
0.7 ± 0.1 od 1	13.9 ± 0.3	15.9 ± 0.7	24.0 ± 2.2	66.5 ± 1.2
1.0 ± 0.2 od 2	14.1 ± 0.4	16.4 ± 0.4	23.6 ± 2.7	67.8 ± 1.2
0.3 ± 0.2	13.6 ± 0.1	15.6 ± 1.5	22.7 ± 3.3	68.1 ± 1.7
2.7 ± 1.2 od 1	3.9 ± 1.0	7.1 ± 1.6	—	—
2.6 ± 0.7 od 2	3.6 ± 1.2	7.8 ± 1.7	—	—
1.3 ± 0.5	2.6 ± 0.6	7.9 ± 0.8	—	—
1.2 ± 1.0	7.4 ± 1.0	9.3 ± 0.8	19.7 ± 1.1	48.3 ± 1.5

Method 1: according to ZELLER⁷.

Method 2: according to FISKE AND SUBBAROW⁸.

DISCUSSION

The fact that considerable amounts of high-energy phosphates were found in the rat uterus under our experimental conditions confirm MENKES AND CSAPO's⁴ views on the fragility of high-energy phosphates in the uterus and the fact that separating the myometrium from the endometrium is fatal to the phosphocreatine and ATP contents of this muscle.

It is clear that the increase of energy-rich phosphates under the influence of oestradiol reflects the overall increase of the uterine metabolism by this hormone and must be correlated with the increased turnover of ATP found by BORELL². The increase of energy-rich phosphates which has been demonstrated in the present work is thus more striking as it is known that oestradiol increases the activity of ATPase⁹ and other phosphatases¹⁰ in the uterus, which would tend to lower the high-energy phosphate content in this muscle. It must also be considered that oestradiol provokes a rise in the water content of the uterus^{11,12}; hence the increase of high-energy phosphates with respect to dry weight would still be higher than that reported in the present paper. MENKES AND CSAPO⁴ have not been able to demonstrate an increase of ATP-like esters throughout the sexual cycle of the rabbit. This may partly be due to the fact, that the doses of oestradiol which have been used in the present

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work are quite high, although they remain within the range of the doses used by WALAAS AND WALAAS¹ and by BORELL^{2,3}.

The progesterone effect on oestradiol-injected immature rats might be correlated with the decreased contractile activity of the uterine muscle under the influence of progesterone¹³ although it has been recently claimed that progesterone injections were unable to diminish the electrical activity in the rat uterus¹⁴. It is interesting to remember that MENKES AND CSAPO⁴ found an increase of phosphocreatine in pregnant rabbit uteri.

In the case of fully mature adult rats the energy-rich phosphate level in the uterus is rather low. The increase of this level in ovariectomized adult animals might be due to the fact that in the uterus of ovariectomized animals phosphate-splitting activities are very low. It is further noteworthy that the phosphate fractions in the uterus of an immature rat differ from the phosphate fractions of an adult ovariectomized rat, especially with respect to phosphocreatine-like esters. This must be kept in mind if one attempts to compare the effect of hormone injections on immature rats or adult ovariectomized rats.

N.B. Since this paper was sent to the editor, further experiments proved, quite surprisingly, that an uterus, which has not been frozen in liquid nitrogen but kept at 0°, shows definitely higher values of energy-rich phosphates than a frozen one. However, the changes induced by oestradiol or progesterone remain the same. Further investigations on this problem are in progress.

SUMMARY

Various fractions of acid-soluble phosphates in the rat uterus were investigated. Oestradiol was found to increase greatly the high-energy phosphate content of the immature rats uterus. Progesterone provoked a further significant increase of the phosphocreatine-like fraction, as compared with the rat treated with oestradiol only. The influence of ovariectomy on the acid-soluble phosphate fractions of the adult rat was likewise studied. The physiological significance of the results is discussed.

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