Regulatory Effect of Energy-Rich Substrates on Phosphorylase *a* Activity in Isolated Rat Uteri[1] (36214)

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When uteri from estrogen-primed ovariectomized rats are isolated in a medium containing glucose, the level of phosphorylase *a* activity is significantly less than in uteri isolated in the medium without glucose (1). The depression of phosphorylase *a* activity by glucose was later demonstrated in the isolated ductus deferens of castrated rats (2) and the rat diaphragm (3). Whether other sugars and some energy-rich substrates, *i.e.*, lactate and pyruvate, could also influence phosphorylase *a* activity in isolated uteri has been investigated and the results are now reported. No exhaustive testing with all possible substrates and with varying concentrations was made in this preliminary study.

Methods. Adult Long-Evans rats were ovariectomized and 7 days later were injected with 50 μg estradiol benzoate, sc. After 48 hr, the rats were anesthetized with sodium pentobarbital (60 mg/kg, ip) and the uterine horns were removed and trimmed separately. The horns from each uterus were suspended by means of hooks and weights in separate chambers containing Krebs-Ringer bicarbonate medium, pH 7.3, at 37° and gassed with 95% O_2 : 5% CO_2. Each chamber with a capacity of 33 ml accommodated 3 horns and one of the chambers contained the substrate to be tested. After 10 min of incubation, the horns were transferred to fresh baths for an additional 50 min. Further details in preparing the organs are reported elsewhere (4). At the termination of incubation, the uterine horns were quickly frozen between smooth blocks of solid CO_2 before the hooks and weights were cut free. Each horn was sealed in parafilm and stored at −90° until assayed for phosphorylase. The concentrations of the substrates were: 11 m*M* for D-glucose, D-mannose, D-galactose, and D-fructose; 13 m*M* for L-arabinose; 10 m*M* for sucrose; 22 m*M* for sodium lactate and sodium pyruvate.

The assay methods for phosphorylase *a* activity (without added adenylic acid) and total phosphorylase activity (with adenylic acid) were previously reported (5). Phosphorylase *a* activity is given as μmoles P_i liberated from glucose-1-PO_4 by 100 mg tissue in 30 min at 37° and as a percentage relative to the total enzyme activity, ± SE of the mean.

Results and Discussion. The levels of phosphorylase *a* activity in uterine horns which were incubated in the medium containing glucose, mannose and galactose were significantly lower than in the control horns (Table I). Fructose and arabinose had no effect on the enzyme activity. Sucrose which does not penetrate the cells did not effect the enzyme activity as might be expected and served as a control for the test system. It should be noted that in the rat uterus, practically all of the phosphorylase and glycogen is localized in the myometrium.

Uteri incubated with pyruvate and lactate also had levels of phosphorylase *a* activity lower than those in the control. Lactate was less effective than pyruvate and the result was significant only when phosphorylase *a* was expressed as a percent of total activity. There were no significant differences in the levels of total phosphorylase in the experimental and control uterine with all substrates tested.

Changes in glycogen levels have been observed in estrogen-primed rat uteri under experimental conditions similar to those reported in this study (4). An initial rapid loss of glycogen occurs during the first 15 min after the uteri are in the bath, either in the presence or absence of glucose or lactate (concentrations

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TABLE I. Phosphorylase *a* Activity in Estrous Rat Uteri *in vitro*: Effect of Energy-Rich Substrates.

Substrate	No. of rats	With Substrate Phosphorylase		Without Substrate Phosphorylase	
		a (μmol P_i)	ratio ($a/t \times 100$)	*a* (μmol P_i)	ratio ($a/t \times 100$)
Glucose	9	5.2 ± 0.3^a	25.4 ± 1.0^a	6.7 ± 0.4	33.7 ± 1.4
Mannose	12	5.5 ± 0.3^a	26.4 ± 1.2^a	7.3 ± 0.3	35.6 ± 0.9
Galactose	6	4.4 ± 0.3^a	28.3 ± 1.5^a	6.4 ± 0.8	35.4 ± 1.6
Fructose	10	6.0 ± 0.4	31.1 ± 0.6	6.4 ± 0.6	31.2 ± 1.4
Arabinose	6	7.6 ± 0.4	37.6 ± 1.8	6.6 ± 0.3	33.5 ± 1.4
Sucrose	6	7.6 ± 0.9	35.5 ± 2.0	7.2 ± 0.6	34.0 ± 1.6
Na pyruvate	6	4.5 ± 0.2^a	27.5 ± 0.6^a	6.0 ± 0.5	36.3 ± 1.5
Na lactate	11	6.5 ± 0.3	28.0 ± 1.0^a	7.0 ± 0.3	32.7 ± 0.5

[a] Difference of means within each experiment $p < .05$; other differences not significant.

11 m*M* and 22 m*M*, respectively). Thereafter, glycogen loss continues but at a rate slower than initially in the absence of substrate. However in the presense of these substrates, the glycogen level remains essentially unchanged for up to 2 hr and does not return to the original concentration. The effect of lactate in lowering phosphorylase *a* activity was not as great as might be expected from its inhibitory effect on glycogenolysis. This could not be correlated with any change in the contractility of the uteri for none of the substrates altered the observed spontaneous contractions. The uteri were always removed from the bath for freezing when they were in a relaxed state to circumvent the effect of contraction on the activity levels of the enzyme.

The effect of sugars upon the level of phosphorylase *a* activity has been studied in the isolated rat diaphragm and the results showed that only glucose among the sugars tested (which were the same as those in the experiments with the uterus) depressed the enzyme activity below the control level (3). Mannose and galactose which were effective in the uterus at 11 m*M* concentration failed to act in the diaphragm at 16 m*M*. Glucose-1-PO_4 and glucose-6-PO_4 also lowered the enzyme activity in the isolated diaphragm (3). It would appear that in order for an energy-rich substrate to regulate phosphorylase *a* activity, it not only must penetrate the muscle cell but also must be capable of being completely metabolized. The isolated uterus metabolizes mannose completely (6) and presumably pyruvate and lactate in the presence of O_2. The metabolism of galactose by the isolated uterus is not known, however.

A mechanism to explain glucose inhibition of phosphorylase *a* activity in the isolated diaphragm has been presented and possibly it could be applicable to explain the observations in the uterus (7). Glucose, glucose-6-PO_4, and glycogen increased the activity of phosphorylase *a* phosphatase prepared from the diaphragm. The phosphatase activity was determined by measuring the rate of conversion of purified muscle phosphorylase *a* to inactive phosphorylase *b*. Phosphorylase *a* activity would be elevated initially because of manipulative stimulation of both tissues in preparation for the bath. Thus either glucose or more likely glucose-6-PO_4 within the cells would increase the formation of inactive phosphorylase at the expense of the active enzyme which would curtail glycogenolysis. To fully apply this explanation to the observations made in the uterus would require a conversion of other effective substrates at least into glucose-6-PO_4 within the cells where it could increase the phosphatase activity. It is also possible that in the uterus all these effective substrates could directly increase the phosphatase activity similar to the effect of glucose on phosphatase in the diaphragm. The mechanism by which glucose and other active energy rich substrates lower the enzyme activity in the uterus remains to be ascertained.

Summary. Estrogen-primed rat uteri isolated

in a Krebs-Ringer bicarbonate bath containing either glucose, mannose, galactose, pyruvate, or lactate had levels of phosphorylase *a* activity significantly lower than in uteri in the bath without these substrates. Fructose, arabinose, and sucrose had no effect on the activity of the enzyme. It appears that the substrate must be capable of complete metabolism by the uterine cells in order to effect the levels of phosphorylase *a* activity. Others report that in the isolated diaphragm, only glucose, among these sugars tested, has an inhibitory effect on the enzyme activity.

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