THE EFFECT OF OESTROGEN ON THE ACTIVITY AND BINDING OF MULTIPLE FORMS OF HEXOKINASE IN THE RAT UTERUS

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The effect of oestrogen on the activity and binding of hexokinase types I and II in the rat uterus has been studied. It was found that 4 hr after administration of oestradiol there was no change in the total tissue content of hexokinase; however, there was a change in the distribution between the particulate and soluble fraction, with an 80% increase in the amount of hexokinase type I bound to the particles. There was no change in the binding of hexokinase type II. Significant increases in the total hexokinase of the uterus were found at 12 hr. At 96 hr hexokinase type I had risen 3-fold in the soluble fraction and 15-fold in the particulate fraction; hexokinase type II increased 10-fold in both fractions.

The administration of oestradiol to immature or ovariectomised rats has been shown to increase the glucose utilization by the uterus. Glycogen synthesis, the incorporation of glucose into CO_2, lipid and RNA are all increased and there is evidence for an increase in the activity of the pentose phosphate pathway (1-6). The hexokinase activity of the uterus has been studied in several laboratories as a possible site for early hormone action since this enzyme is key to the entry of glucose into any of these pathways (4,7,8). It has been shown that oestradiol increases the soluble hexokinase activity of the immature or ovariectomised rat uterus, the earliest statistically significant change being found 8 hr after administration of the hormone (7,8); these authors have pointed out that this effect occurs too late to be a primary event in the early stimulation of glucose metabolism (6-8).

Recent studies have shown that hexokinase is subject to a wide variety of control mechanisms, particularly with respect to the binding of the enzyme to mitochondria (9,10); this binding modifies such factors as glucose 6-phosphate inhibition and the K_m for ATP (10-12) and it is possible

that by this means effective control of hexokinase may be mediated by a process other than that of increased enzyme synthesis. It therefore seemed of interest to examine the early effects of oestradiol on the distribution of hexokinase types I and II between the soluble and particulate fraction of the cell in rat uterus.

MATERIALS AND METHODS

Immature female rats, 3 weeks old, weighing between 40 - 50 g, were used. 17β-Oestradiolbenzoate (5 mg in 1 ml arachis oil) was obtained from BDH and was diluted with ethanol to give a solution of 50 μg/ml. Rats received intraperitoneal injections of 10 μg/100 g body wt. and were killed subsequently at the stated times; the immature controls were treated with the equivalent volume of alcohol/arachis oil solution. The uteri from 3 to 5 rats were pooled to give sufficient material for one fractionation, each experimental group containing not less than 8 such values. The uteri were excised, chopped finely and then homogenised (using a Potter homogeniser with a teflon plunger) in 20 volumes of ice-cold medium containing 150 mM-KCl, 5 mM-$MgCl_2$, 5 mM-EDTA and 0.1 mM-dithiothreitol, using a standardised number of strokes and time of homogenising (2 min). The homogenate was centrifuged at 700 g for 10 min to separate the debris and nuclei, the supernatant was then centrifuged at 105,000 g for 45 min and the residue designated the particulate fraction, the high speed supernatant fraction was designated the soluble fraction. The pellet was rinsed with the homogenising medium and then suspended with gentle hand homogenisation in a known volume (about 2 - 3 ml) of the same medium. Both fractions were dialysed for 2 hr in the cold. The total hexokinase (ATP:D-hexose 6-phosphotransferase, EC 2.7.1.1) was measured as previously described, using a glucose concentration of 5 mM (13,14). Heat treatment at 45° for 1 hr was used to distinguish hexokinase types I and II (14,15). No glucokinase could be detected in these preparations.

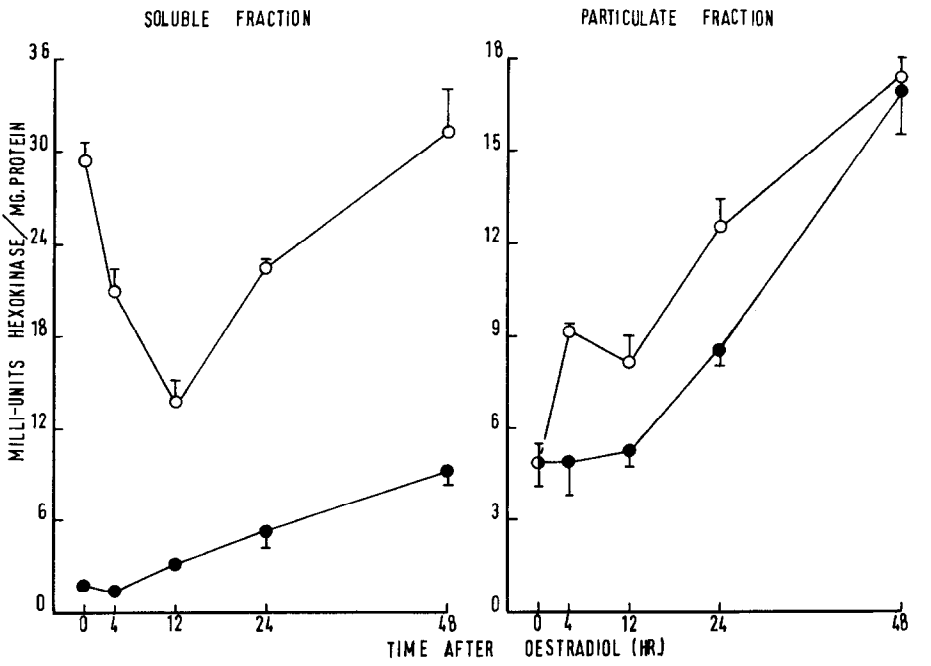

Figure 1. The effect of oestradiol on the activity of hexokinase/mg protein in the soluble and particulate fraction of the rat uterus. The results, as milliunits/mg protein, are the mean of 8 values, the vertical lines representing the SEM values shown in one direction only.

○——○ Hexokinase type I , ●——● Hexokinase type II

RESULTS

The changes in hexokinase activity and distribution between the soluble and particulate fraction of the whole uterus at different times after oestradiol treatment are shown in Figure 1 and Table 1. The immature rat uterus is characterised by a very low proportion of bound hexokinase. At 4 hr after administration of oestradiol there was a significant fall in hexokinase type I of the soluble fraction and a parallel rise in the particulate bound form of this enzyme. The protein content of the particulate fraction did not increase significantly at this time of treatment, suggesting that there was an increased binding of hexokinase to the existing particulate fraction under the influence of oestradiol rather than an increase in the amount of the particulate fraction. Hexokinase type II

TABLE I

THE EFFECT OF OESTRADIOL TREATMENT ON THE ACTIVITIES OF HEXOKINASE TYPE I AND TYPE II IN THE SOLUBLE AND PARTICULATE FRACTION OF THE UTERUS

Time after oestradiol administration (hr)	Uterus wt. (mg)	Protein content (mg/uterus)		Hexokinase (milliunits/total uterus)			
				Soluble fraction		Mitochondrial fraction	
		soluble	mitochondrial	Type I	Type II	Type I	Type II
0	25 ± 1	0·82 ± 0·05	0·36 ± 0·03	26·6 ± 0·8	1·53 ± 0·12	2·08 ± 0·28	2·10 ± 0·27
4	38 ± 4	1·10 ± 0·04	0·36 ± 0·03	22·8 ± 0·8	1·80 ± 0·22	3·72 ± 0·25	2·10 ± 0·54
		P <0·001	N.S.	P <0·01	N.S.	P <0·001	N.S.
12	46 ± 3	1·93 ± 0·13	0·72 ± 0·03	25·3 ± 1·5	5·92 ± 0·68	5·62 ± 0·41	3·81 ± 0·55
		P <0·001	P <0·001	N.S.	P <0·001	P <0·001	P <0·05
24	60 ± 3	2·47 ± 0·13	1·21 ± 0·11	55·1 ± 3·8	12·7 ± 2·6	14·7 ± 1·0	10·3 ± 1·0
48	93 ± 4	2·65 ± 0·31	1·78 ± 0·26	76·7 ± 2·3	22·5 ± 1·4	30·3 ± 4·0	25·5 ± 2·0
96	83 ± 6	3·16 ± 0·21	1·45 ± 0·13	78·4 ± 3·7	15·2 ± 3·0	34·8 ± 5·1	22·7 ± 2·5

A single dose of 17β-oestradiol benzoate (10 μg/100 g body wt.) was given intraperitoneally and the rats killed at the stated time intervals. The results are all given on the basis of total organ weight, each group contained not less than 8 values. The values are the means ± SEM. Fisher's P values are given for the comparison of the groups treated with oestradiol at 4 hr and 12 hr with the immature control group. Values greater than 0.1 are quoted as N.S., not significant. At 24, 48 and 96 hr after administration of oestradiol all values are highly significantly different from the immature control group (P <0.001).

remains completely unchanged in the soluble and particulate fraction of the cell at this time after oestradiol administration.

At 12 hr after administration of oestradiol there was a significant rise in the hexokinase of the uterus in both the soluble fraction (a 4-fold rise in hexokinase type II) and in the particulate fraction (a 2-fold rise in hexokinase type I and type II). On the basis of activity/mg protein there was an apparent fall in the soluble fraction hexokinase type I as a result of a 2-fold increase in protein content of this fraction.

Between 12 and 48 hr there is an almost linear increase in hexokinase types I and II in both the soluble and particulate fractions. The pattern established at 48 hr, and remaining almost unchanged at 96 hr, is (on the basis of total activity in the uterus) a 3-fold increase of hexokinase type I in the soluble fraction and a 15-fold increase in the particulate fraction; hexokinase type II increased 10-fold in both cell fractions. On the basis of hexokinase activity/mg protein, the soluble fraction shows a rise in the type II with no change in the type I enzyme, while in the particulate fraction hexokinase type I and type II show very similar increases.

DISCUSSION

The earliest change observed in the present experiments (4 hr after oestradiol administration) was an increase in hexokinase type I of the particulate fraction with a concomitant fall in that of the soluble fraction, suggesting the possibility that binding of hexokinase type I might be critical in the early stages of control of glucose phosphorylation; hexokinase type II does not seem to be involved at this stage. This is in line with the view put forward by Katzen (16) that the presence or absence of type I hexokinase may be an important factor in the mediation of insulin action.

Several lines of evidence suggest that binding of hexokinase to mitochondria is an important facet of control of glucose metabolism. It has

been shown that the bound form of hexokinase is less susceptible to inhibition by glucose 6-phosphate in vitro compared with the free form (10-12). Indirect evidence has been obtained from studies of transient state metabolite profiles in ascites cells that in the intact cell hexokinase is able to carry out a rapid rate of glucose phosphorylation in the presence of inhibitory concentrations of glucose 6-phosphate and that cessation of the rapid rate coincides with the known time required for elution of hexokinase from mitochondria by glucose 6-phosphate (9,17). There is also the possibility of involvement of bound hexokinase in the direct shuttle of ADP from the hexokinase system to the respiratory chain of the mitochondria (9,18,19). Early effects of insulin on binding of hexokinase to mitochondria have been reported for mammary gland and adipose tissue (20,21). Finally, there appears to be some specificity in the binding with respect to the relative amounts of hexokinase type I/type II in the two cell fractions of the uterus. Throughout the period of treatment there was a predominance of hexokinase type I in the soluble fraction but approximately equal amounts of the two forms in the particulate fraction.

While in the early stages of oestradiol treatment the emphasis is on the changes in hexokinase type I, the later stages are characterised by changes in both forms of the enzyme. The present changes are in accord with the results of Smith and Gorski (7) and of Valadares, Singhal and Parulekar (8), who showed significant increases of soluble fraction hexokinase 8 hr after oestradiol treatment.

The question arises as to whether the increased binding of hexokinase type I occurs sufficiently rapidly after oestradiol administration to account for the changes in glucose utilization. This appears to be at least as rapid as changes in phosphofructokinase, glucose 6-phosphate dehydrogenase and phosphoglucose isomerase (2,22,23). However, changes in the rate of $^{14}CO_2$ and ^{14}C-lipid formation from specifically labelled glucose have been found 2 hr after oestradiol treatment, hence the period of incubation of 1-2

hr must not be ignored (2,6). One of the most rapid responses to oestradiol is the increase in nuclear RNA synthesis which occurs within minutes rather than hours (24); there is a fall in ATP during this period (26). The increase in bound hexokinase, which has a lower K_m for ATP, takes on added significance in the light of the 50% fall in this nucleotide during the 4 hr following oestradiol administration (26).

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