THE EFFECT OF OESTRADIOL ON THE PROFILE OF CONSTANT AND SPECIFIC PROPORTION GROUPS OF ENZYMES IN RAT UTERUS

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SUMMARY: Enzymes of the pentose phosphate pathway, the glycolytic pathway, the tricarboxylic acid cycle and fatty acid synthesis were measured in immature rat uterus before, and 16 hr after, treatment with oestradiol. The flux of glucose through alternative pathways was examined. The changes observed were evaluated on the basis of constant and specific proportionality groupings. Coordinated increases were found among enzymes of the glycolytic and tricarboxylic acid sequence of enzymes. Specific proportion increases were found in particulate bound hexokinase, in G6P dehydrogenase and R5P isomerase, changes which may be correlated with the increased lipid synthesis and nucleic acid synthesis occurring following oestradiol treatment.

INTRODUCTION

That there is a basic framework of enzyme activity upon which is superimposed a distinctive pattern related to the particular metabolic pathways of the tissue, has been revealed by a study of constant and specific proportion groups of enzymes [1]. This principle has been used both in comparisons among different tissues [2,3] and in the investigation of specialised tissues such as mammary gland and adipose tissue in different functional conditions [4], where this approach has been valuable in differentiating between sources of cytosolic NADPH for reductive synthetic reactions.

Many of the studies of uterine response to oestradiol have been concerned primarily with the sequence and absolute magnitude of change of enzymes. In general, progressive increases have been found in the enzymes investigated [5-8]. The present study attempts to examine the changes in the enzymes of the glycolytic sequence, the pentose phosphate pathway and certain of those of the tricarboxylic acid cycle and fatty acid synthesis in uterus following oestradiol treatment, on the basis of the concepts of constant and specific proportionality, with a view to determining which enzymes increase in parallel, and thus form the basic framework, and which increase faster than the basic group, and thus may have a special role in the hormonal response of the tissue.

METHODS

Animals: Immature female rats, 3 weeks old, weighing between 40−50 g were given intraperitoneal injections of 17β-oestradiol benzoate (10μg/100g body wt)

or the equivalent volume of solvent medium, and were killed 16 hr later. The uteri from 15 rats were pooled for each control value and from 10 rats for each oestradiol-treated value. Each group contained not less than 6 such values.

Preparation of homogenates: Homogenates were prepared (using a Potter homogenizer with Teflon plunger) in 9 vol. ice cold medium containing 150mM-KCl, 5mM-$MgCl_2$, 5mM-EDTA and 0.1mM dithiothreitol. The fractionation procedure was as described previously [9].

Estimation of phosphofructokinase (PFK): Kono & Uyeda [10] have recently shown that PFK is cold labile and that protein concentration, pH and ionic composition of the medium are all critical to the stability of this regulatory enzyme. At protein concentrations of 5mg/ml, or above, the enzyme is stable at 0° for 1 day [10].

Preliminary experiments using dilute homogenates of rat uterus, prepared in 30 volumes of medium, gave low values for PFK activity (see Table 1). It appeared that dilution caused a more marked inactivation of PFK of the control group than of the oestradiol-treated group, thus a falsely exaggerated view of the effect of oestratiol treatment on PFK activity might be observed under these conditions. The present modification of preparing homogenates in 9 volumes of medium, thus raising the protein concentration of the supernatant fraction to a value close to 5mg/ml, is of critical importance in assessing changes in PFK activity (Table 1).

All other enzymes were estimated as described previously [11]. The results are given as means ± SEM.

RESULTS AND DISCUSSION

The results in Table 2 show that oestradiol treatment increases the total glucose utilisation of immature rat uterus and that, of the glucose utilised, a greater proportion is metabolised via the pentose phosphate pathway. There is a parallel rise in the oxidation of glucose by the pentose phosphate pathway and the incorporation into lipid. These results are in accord with those of Barker *et al.* [13,14].

The enzyme profiles of immature rat uteri before and after treatment with oestradiol are shown in Table 3. This records activity per uterus and also gives the quotient of activity 16 hr after oestradiol treatment/activity of immature control group. This quotient is of importance in delineating which enzymes have an outstanding rate of increase and constitute a specific proportion group. A similar quotient is given in Table 2 for the overall flux of glucose through alternative pathways.

The majority of the enzymes of the glycolytic pathway behave as a constant proportion group showing increases of approximately two-fold with the notable

Table 1. Effect of protein concentration on the estimated activity of phosphofructokinase in rat uterus.

	Control	Oestradiol-treated (16 hr)
A. *Dilute homogenates (1:30)*		
No. of uteri/extract	5	3
Protein content of soluble fraction (mg/ml)	1.6±0.2	1.8± 0.3
PFK activity (munits/uterus)	5.5±0.8	54 ± 7.7
B. *Concentrated homogenates (1:10)*		
No. of uteri/extract	15	10
Protein content of soluble fraction (mg/ml)	4.5±0.3	5.9± 0.3
PFK activity (munits/uterus)	66 ±4	127 ±17

Table 2. Effect of oestradiol on the flux of glucose through alternative pathways of glucose metabolism.

	Control	Oestradiol-treated (16 hr)	Oestradiol / Control
	mμmoles/uterus/hr		
1. Total glucose utilised	188 ±15	498±76	2.6
2. Oxidation via pentose phosphate pathway	21 ± 2	126±21	6.0
3. Incorporation into lipid	9.3± 1.6	62±15	6.7
4. Metabolism via glycolytic route	117 ±12	256±42	2.2
5. % Contribution of pentose phosphate pathway to glucose metabolism	4.5%	11.0%	

1. Calculated from the incorporation of uniformly labelled glucose into CO_2, lipid, protein and lactate.
2. Calculated from the formation of $^{14}CO_2$ from [1-^{14}C]glucose - $^{14}CO_2$ from [6-^{14}C]glucose. No correction was made for low rate of recycling as shown by formation of $^{14}CO_2$ from [2-^{14}C]glucose.
3. Calculated from the incorporation of [U-^{14}C]glucose into lipid.
4. Calculated from the incorporation of [3,4-^{14}C]glucose into CO_2.
5. Calculated from the specific yields of $^{14}CO_2$ from [1-^{14}C]glucose and [6-^{14}C]glucose by the method of Katz *et al.* [12] assuming equilibration of triose phosphates.

Table 3. The effect of oestradiol on the enzyme profile of rat uterus.

	Control	Oestradiol-treated (16 hr)	Oestradiol / Control
	munits/uterus		
GLYCOLYTIC PATHWAY			
Hexokinase, cytosolic	24.1± 1.8	55.2± 4.2	2.3
particulate	3.7± 0.5	22.7± 1.9	6.1 †
Phosphoglucomutase	139 ± 12	196 ± 31	1.4
Phosphoglucose isomerase	348 ± 26	940 ±110	2.7
Phosphofructokinase	66 ± 4	127 ± 17	1.9
Fructose diphosphatase	9.0± 0.9	13.1± 1.2	1.5
Aldolase	54 ± 1.7	114 ± 2	2.1
Triosephosphate isomerase	261 ± 26	326 ± 92	1.3
Glycerol 1-P dehydrogenase	16.7± 5.8	18.3± 5.6	1.1
GAP dehydrogenase	137 ± 22	153 ± 29	1.1
Phosphoglycerate kinase	22 ± 3	40 ± 7	1.9
Phosphoglyceromutase	69 ± 16	96 ± 12	1.4
Enolase	26 ± 3	61 ± 10	2.4
Pyruvate kinase	428 ± 31	790 ± 66	1.9
Lactate dehydrogenase	401 ± 43	871 ± 74	2.2
PENTOSE PHOSPHATE PATHWAY			
G6P dehydrogenase	18.2± 1.4	94.8± 16	5.2 †
6PG dehydrogenase	12.0± 0.5	33.6± 2.6	2.8
R5P isomerase	63 ± 5	272 ± 18	4.3 †
Ru5P epimerase	236 ± 35	609 ± 27	2.6
Transketolase	7.8± 0.5	16.5± 0.9	2.1
Transaldolase	29 ± 1.0	37 ± 2.3	1.3
FATTY ACID SYNTHESIS and RELATED ENZYMES			
Citrate synthase	8.4± 0.9	18.4± 1.0	2.2
Citrate cleavage enzyme	3.4± 0.2	7.5± 0.4	2.2
Fatty acid synthetase	1.6± 0.1	3.5± 0.4	2.2
G6P dehydrogenase	18.2± 1.4	94.8± 16	5.2 †
ICDH ($NADP^+$), cytosolic	69 ± 0.7	93 ± 4	1.3
Malic enzyme ($NADP^+$)	5.1± 0.6	8.5± 0.4	1.7
MDH (NAD^+), cytosolic	1453 ±108	2448 ±154	1.7
Pyruvate carboxylase	6.9± 0.2	11.5± 0.1	1.7
TRICARBOXYLIC ACID CYCLE and RELATED ENZYMES			
Citrate synthase	8.4± 0.9	18.4± 0.9	2.2
ICDH ($NADP^+$), particulate	27 ± 1.2	69 ± 1.2	2.5
MDH (NAD^+), particulate	213 ± 9	517 ± 5	2.4
Glutamate dehydrogenase	23 ± 1	45 ± 2	2.0
GOT, cytosolic	121 ± 25	236 ± 54	1.9
particulate	56 ± 4	126 ± 15	2.3
GPT, cytosolic	15 ± 0.8	30 ± 3	2.0
particulate	7.3± 0.8	13.1± 1.6	1.8
Weight of uterus (mg.)	20.9± 0.9	48.2± 2.5	2.3

† Indicates enzymes showing 'specific proportion' increases in activity with oestradiol treatment.

exception of particulate bound hexokinase which increases six-fold. The parallelism between the increase in total glucose utilisation, the flux of glucose through the glycolytic pathway and the activities of enzymes of the glycolytic pathway is striking. Special attention should perhaps be drawn to the constant proportionality of PFK in the glycolytic sequence. Reports have been made of very marked increases in the activity of this enzyme in oestradiol-treated rats [7] but, as shown in Table 1, these differences may be ascribed to the conditions of extraction, notably the protein concentration which has a profound effect on the stability of the enzyme [10].

Of the enzymes of the pentose phosphate pathway, G6P dehydrogenase shows a five-fold increase following oestradiol treatment which may be compared with the flux through the pentose phosphate pathway and the rate of lipogenesis which increase approximately six-fold. Several enzymes linked to lipogenesis were measured in the present study (Table 3) and all showed constant proportionality increases of approximately two-fold. It might be anticipated that acetyl CoA carboxylase, not measured in this study, could behave as a specific proportion enzyme in view of its role in the control of lipogenesis.

Specific proportion changes in G6P-dehydrogenase have been observed in a number of lipogenic situations, in mammary gland at different stages of the lactation cycle [4] and in liver when a high carbohydrate diet is administered following a period of starvation [15,16]. Such "overshoot" or specific proportion changes may play a vital role in directing G6P into the pathway most efficient for the production of NADPH.

An unusual finding, in the context of constant and specific proportionality, is that R5P isomerase increases faster than the remaining non-oxidative enzymes of the pentose phosphate pathway. This might effectively direct ribulose 5-phosphate towards ribose 5-phosphate formation thus favouring nucleotide synthesis. It has been shown that an early action of oestrogens on immature rat uterus is the increased uptake of labelled precursors into RNA [17,18] and here it may be noteworthy that a 50% increase in R5P isomerase was observed as early as 2 hr after administration of oestradiol.

The possibility that changes in R5P isomerase might be important in channeling ribulose 5-phosphate towards RNA synthesis is also supported by the outstanding increase in this enzyme in a situation of marked hypertrophy of the liver as seen in rats bearing a growth hormone secreting pituitary tumour [19]. It thus appears that the specific group changes following oestradiol treatment are in accordance with the concept that control occurs at branch points of metabolism [20].

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REFERENCES

1. Pette, D., in *Regulation of Metabolic Processes in Mitochondria* (Ed. J.M. Tager, S.Papa, E.Quagliariello and E.C.Slater) p.28 (Elsevier Publishing Co., Amsterdam, 1966).
2. Scrutton, M.C., and Utter, M.F., *Ann.Rev.Biochem.* 37, 249 (1968).
3. Bass, A., Brdiczka, D., Eyer, P., Hofer, S., and Pette, D., *European J. Biochem.* 10, 198 (1969).
4. McLean, P., Greenbaum, A.L., and Gumaa, K.A., *FEBS Letters* 20, 277 (1972).
5. Scott, D.B.M., and Lisi, A.G., *Biochem.J.* 77, 52 (1960).
6. Eckstein, B., and Villee, C.A., *Endocrinol.* 78, 409 (1966).
7. Singhal, R.L., Valadares, J.R.E., and Ling, G.M., *J.Biol.Chem.* 242, 2593 (1967).
8. Singhal, R.L., and Valadares, J.R.E., *Amer.J.Physiol.* 218, 321 (1970).
9. Baquer, N.A., and McLean, P., *Biochem.Biophys.Res.Commun.* 37, 158 (1969).
10. Kono, N., and Uyeda, K., *Biochem.Biophys.Res.Commun.* 42, 1095 (1971).
11. Gumaa, K.A., and McLean, P., *Biochem.J.* 115, 1009 (1969).
12. Katz, J., Landau, B.R., and Bartsch, G.E., *J.Biol.Chem.* 241, 727 (1966).
13. Barker, K.L., and Warren, J.C., *Endocrinol.* 78, 1205 (1966).
14. Barker, K.L., Nielson, M.H.N., and Warren, J.C., *Endocrinol.* 79, 1069 (1966).
15. Tepperman, H.M., and Tepperman, J., *Advances in Enzyme Reg.* 1, 121 (1963).
16. Novello, F., Gumaa, K.A., and McLean, P. *Biochem.J.* 111, 713 (1969).
17. Gorski, J., and Nicolette, J.A., *Arch.Biochem.Biophys.* 103, 418 (1963).
18. Billing, R.J., Barbiroli, B., and Smellie, R.M.S., *Biochem.J.* 112, 563 (1969).
19. Gumaa, K.A., MacLeod, R.M., and McLean, P., *Biochem.J.* 113, 215 (1969).
20. Krebs, H.A., and Kornberg, H.L., *Ergebn.Physiol.* 49, 212 (1957).