Pyruvate Disposal by Lactating-Rat Mammary Gland

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The fates of carbon atoms from pyruvate in lactating-rat mammary gland are shown in Scheme 1 (based on the review by Bauman & Davis, 1974). To avoid complicating the scheme we have not indicated that the major source of NADPH required for fatty acid synthesis is the hexose monophosphate shunt. In fact, oxidation of glucose by the first two enzymes of this pathway is limited by the availability of NADP+ regenerated from NADPH by the activity of fatty acid synthetase. It is generally accepted that in non-ruminants, including the rat, the major source of acetyl units in fatty acid synthesized by the mammary gland is plasma glucose via cytosolic pyruvate generated by the joint operation of the Embden–Meyerhof and hexose monophosphate-shunt pathways. Although Hawkins & Williamson (1972) could not detect significant uptake of plasma lactate by the gland in situ, this is a possible alternative source of pyruvate, and both Katz et al. (1974) and Bartley & Abraham (1976) reported that, for lactating mammary parenchymal cells incubated in vitro, considerably higher rates of fatty acid synthesis were achieved when glucose and lactate were supplied compared with glucose alone, and that in the former situation exogenous lactate provided most of the carbon in the synthesized fatty acids.

Regulation by metabolites and hormones

Studies in vitro with isolated mammary cells (Martin & Baldwin, 1971) suggested that insulin was an important regulator of the rate of glucose-carbon incorporation into fatty acids, that its effect was exerted after the formation of glucose 6-phosphate and involved an increase in the cytosolic NAD+/NADH ratio. Williamson et al. (1975) showed that in lactating-mammary-gland slices acetocetate depressed both glucose utilization and oxidation of pyruvate. Insulin reversed the former effect but not the latter; they explained their observations by suggesting that acetocetate inhibited pyruvate dehydrogenase (EC 1.2.4.1) and insulin treatment activated acetyl-CoA carboxylase (EC 6.4.1.2). It is not excluded, however, that under different circumstances insulin may influence other enzymic steps on the pathway from glucose to fatty acids.

Pyruvate dehydrogenase reaction as a site of regulation

Scheme I indicates that, judging from $V_{\text{max}}$, activities of the extracted enzymes, the pyruvate carboxylase (EC 6.4.1.1), pyruvate dehydrogenase and acetyl-CoA carboxylase reactions are all possible sites of rate-limitation for incorporation of carbon atoms from carbohydrate into fatty acids. The value for acetyl-CoA carboxylase activity was, however, determined under conditions which may not be optimal for the enzyme (cf. Halestrap & Denton, 1973). Since pyruvate dehydrogenase is an important regulatory enzyme in other mammalian tissues (see the review by Denton et al., 1975), it seems likely that at least under some conditions the activity of this enzyme may determine the flux of acetyl units from pyruvate to fatty acids. Coore & Field (1974) showed that pyruvate dehydrogenase in rat mammary gland was subject to control by phosphorylation/dephosphorylation, as is the case for the analogous enzyme in other tissues, and that between the end of pregnancy and the peak of lactation the total enzyme activity (after dephosphorylation) increased sevenfold and the proportion of enzyme dephosphorylated...
Scheme 1. *Fates of carbon from lactate or pyruvate in lactating-rat mammary gland*

Numbers beside enzymic steps represent $V_{\text{max}}$, activities of extracted enzymes expressed as $\mu$mol/min per g of tissue corrected for contained fat and milk. Values are given as nearest whole numbers from data of Gumaa *et al.* (1973).

*in vivo* increased threefold. This suggested that the enzyme adaptation could accommodate a flux change of 21-fold, which is similar to the reported increases in extracted activities of other enzymes involved in the pathway from pyruvate to fatty acids.

The data of Bartley & Abraham (1976) show the maximal rate of incorporation of acetyl units from carbohydrate precursors into fatty acids of lactating mammary gland to be $0.95 \mu$mol/min per g wet wt. This is very similar to the activity of extracted pyruvate dehydrogenase if one allows for a temperature correction (30°C assay temperature) and also partial phosphorylation of the enzyme even at the peak of lactation (30-40% inactivated owing to phosphorylation). Since it is well established that at least three hormones, namely prolactin, insulin and cortisol, are necessary for the development of rat mammary gland (Anderson, 1974), we investigated the effect of various hormonal manipulations on the behaviour of pyruvate dehydrogenase in lactating mammary gland on the assumption that these hormones maintain as well as induce the adaptations of the gland. We have thus been concerned with determining the hormonal factors that maintain a rapid flow of carbon atoms through the pathway from pyruvate to fatty acid.
Table 1. Reversal by insulin of the effects of prolactin withdrawal on lactating-rat mammary-gland pyruvate dehydrogenase

Prolactin withdrawal was achieved by subcutaneous injection of 1 mg of 2-bromo-α-ergocryptine 24 h before removal of the gland, and soluble insulin (7.1 u) was injected intraperitoneally 21 h later. Control animals were injected either with the solvent for 2-bromo-α-ergocryptine or with 0.9% NaCl instead of insulin at the appropriate times. In experimental series A the rats were 4 days lactating and in B they were 12 days lactating. Pyruvate dehydrogenase was assayed in extracts of gland freeze-clamped while the animals were under halothane anaesthesia. Results (means ± S.E.M., with numbers of animals in parentheses) are expressed as milliunits (nmol of product formed per min at 30°C) per mg of DNA. ‘Initial’ pyruvate dehydrogenase was measured in extracts containing 5 mM-EDTA; ‘total’ pyruvate dehydrogenase activity was the maximal activity seen after incubation of extracts with 10 mM-MgCl₂ and exogenous pyruvate dehydrogenase phosphatase. *P < 0.05, **P < 0.01, comparing data in any column with that with animals injected only with solvent and 0.9% NaCl. †P < 0.05, ††P < 0.01, comparing data in the same column from animals injected with insulin and 2-bromo-α-ergocryptine with data from animals injected only with the latter.

<table>
<thead>
<tr>
<th>Expt. series</th>
<th>Treatment</th>
<th>'Initial' pyruvate dehydrogenase activity</th>
<th>'Total' pyruvate dehydrogenase activity</th>
<th>'Initial'/'total' pyruvate dehydrogenase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Solvent followed by 0.9% NaCl injection</td>
<td>75 ± 3 (14)</td>
<td>157 ± 19 (14)</td>
<td>0.48 ± 0.04 (14)</td>
</tr>
<tr>
<td></td>
<td>2-Bromo-α-ergocryptine followed by 0.9% NaCl injection</td>
<td>31 ± 4 (17)**</td>
<td>125 ± 8 (17)</td>
<td>0.28 ± 0.06 (17)**</td>
</tr>
<tr>
<td></td>
<td>2-Bromo-α-ergocryptine followed by insulin injection</td>
<td>103 ± 13 (18)††</td>
<td>170 ± 19 (16)†</td>
<td>0.56 ± 0.04 (16)††</td>
</tr>
<tr>
<td></td>
<td>Solvent followed by insulin injection</td>
<td>123 ± 16 (10)**</td>
<td>175 ± 17 (10)</td>
<td>0.69 ± 0.05 (10)**††</td>
</tr>
<tr>
<td>B</td>
<td>Solvent followed by 0.9% NaCl injection</td>
<td>149 ± 34 (7)</td>
<td>280 ± 23 (7)</td>
<td>0.54 ± 0.10 (7)</td>
</tr>
<tr>
<td></td>
<td>2-Bromo-α-ergocryptine followed by 0.9% NaCl injection</td>
<td>43 ± 8 (5)**</td>
<td>209 ± 13 (5)**</td>
<td>0.21 ± 0.04 (5)**††</td>
</tr>
<tr>
<td></td>
<td>2-Bromo-α-ergocryptine followed by insulin injection</td>
<td>248 ± 31 (8)††</td>
<td>382 ± 48 (8)††</td>
<td>0.65 ± 0.05 (8)††</td>
</tr>
<tr>
<td></td>
<td>Solvent followed by insulin injection</td>
<td>219 ± 30 (6)</td>
<td>317 ± 53 (6)</td>
<td>0.71 ± 0.05 (6)††</td>
</tr>
</tbody>
</table>

Factors influencing pyruvate dehydrogenase activity in vivo and in vitro

Earlier experiments (Field & Coore, 1976) showed that withdrawal of either prolactin or insulin for as little as 3 h led to increased phosphorylation and hence inactivation of the enzyme in vivo. Decrease in the amount of circulating prolactin was achieved by injection of 2-bromo-α-ergocryptine (Seki et al., 1974) and of circulating insulin by injection of streptozotocin (Schein et al., 1971). In mid- and late-lactation replacement dosage of the appropriate hormones counteracted the effect on the enzyme of the injected drugs. At 4 days lactation, however, insulin but not prolactin was able to correct the effect of dosage.
with 2-bromo-α-ergocryptine. Table 1 shows some further experiments investigating this phenomenon. At both 4 and 12 days of lactation, insulin injection (given 21 h after 2-bromo-α-ergocryptine and 3 h before freeze-clamping of the gland and extraction of the enzyme) was able to increase 'initial' activity of pyruvate dehydrogenase to values higher even than the controls which has not been exposed to either agent. Furthermore there was a tendency for the 'total' extractable pyruvate dehydrogenase activity to fall after 2-bromo-α-ergocryptine treatment, and this was corrected by insulin treatment. In fact, in 4- and 12-day-lactating rats there was a significant increase in 'total' enzyme activity in rats receiving insulin and 2-bromo-α-ergocryptine compared with those receiving only the latter. No complete explanation for these observations is available. The fact that animals treated with 2-bromo-α-ergocryptine showed somewhat higher concentrations of plasma glucose and non-esterified fatty acids than controls at the time of operation (Field & Coore, 1976) might suggest that the drug had decreased the amount of circulating insulin, but measurements of plasma insulin have not so far confirmed this suggestion. An alternative possibility is that the drug stimulated production of another hormone antagonistic in action to insulin, and adrenal steroids are obvious candidates. However, injection of large doses of cortisol into 4-day-lactating rats was without effect on the degree of activation of mammary-gland pyruvate dehydrogenase and did not disturb the effect of injected insulin in increasing the 'initial' activity of the enzyme above control values (cf. Table 1). The possibility that the effects of injections of 2-bromo-α-ergocryptine and of insulin both depend on the concentration of plasma non-esterified fatty acids has been considered, but also seems an inadequate explanation (Field & Coore, 1976).

Attempts have been made to detect hormonal effects on pyruvate dehydrogenase in lactating mammary tissue incubated in vitro, but these have been frustrated by a tendency for the 'total' pyruvate dehydrogenase activity to fall and the degree of activation of the enzyme to rise during 2h incubation of acini prepared by the method of Katz et al. (1974). It seemed that neither Krebs medium nor Medium 199 nor even serum from lactating rats was able to provide conditions guaranteeing stability in behaviour of the enzyme complex during incubations in vitro lasting more than 1 h.

As stated above, Williamson et al. (1975) concluded from indirect evidence that inclusion of acetoacetate in the incubation medium of mammary-gland slices inhibited pyruvate dehydrogenase within the tissue. This may not, however, involve increased phosphorylation of the enzyme but only product inhibition by acetyl-CoA. In similar experiments we have not so far detected an effect of acetoacetate on the degree of phosphorylation of pyruvate dehydrogenase extracted from such incubated tissue. It may be that a complex interplay of variable metabolite supply and hormonal influences underlies the effects on the enzyme in vivo.

Properties of isolated mammary-gland mitochondria

We also explored an alternative approach in which we do not ask what factors external to the mammary-gland cells are modifying pyruvate dehydrogenase activity, but rather what factors in the immediate vicinity of the enzyme could directly influence its activity. To this end we prepared well-coupled mitochondria from lactating mammary gland and begun to examine some of their properties (Titheradge & Coore, 1977). In particular we have measured the maximum rate of pyruvate transport in these mitochondria, since it is conceivable that the availability of pyruvate in the matrix controls the degree of phosphorylation of the enzyme (Coore & Field, 1974). However, even in the case of streptozotocin-diabetic rats, where the pyruvate dehydrogenase was very largely (80–100%) inactivated by phosphorylation, we could find no impairment of pyruvate transport by isolated mitochondria under the particular conditions selected. We did, however, detect one interesting characteristic of mammary-gland mitochondria which may be relevant to their handling of pyruvate. It would appear that transport of pyruvate in any direction across the mitochondrial membrane is favoured by the passage of malate or citrate, but not by phosphate or α-oxoglutarate in the opposite direction. A linkage of anion transport in this way makes physiological sense in relation to the pathways
shown in Scheme I. Entry of pyruvate into the mitochondria is associated with the exit of malate and citrate. Thus both acetyl units and reducing power are diverted from the tricarboxylic acid cycle out into the cytosol, where they are used in lipogenesis.

Interference with this anion exchange by deprivation of insulin or prolactin could conceivably account for some of our results, since Taylor & Halperin (1973) have argued that intramitochondrial accumulation of citrate can impair the activity of pyruvate dehydrogenase phosphatase and thus inactivate pyruvate dehydrogenase.

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Human Milk Fucosyltransferases

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Human milk contains numerous oligosaccharides derived from lactose. The structures of many of them have been determined (Kobata, 1972), and they have proved useful in studies involving glycosyltransferases and glycosidases. The presence of these oligosaccharides in individuals is a function of the blood type of the donor. This is also the case for the specific glycosyltransferases responsible for their syntheses.

Fucose has been found to be involved in four different linkages in human milk oligosaccharides: $\alpha1\rightarrow3$ and $\alpha1\rightarrow2$ linkages with galactose (Yamashita et al., 1976); $\alpha1\rightarrow3$ and $\alpha1\rightarrow4$ linkages with N-acetylglucosamine and glucose when these monosaccharides are themselves bound in an $\alpha1\rightarrow4$ or $\alpha1\rightarrow3$ linkage with galactose.

$\alpha1\rightarrow2$ and $\alpha1\rightarrow4$ fucosyltransferases have already been the subject of several reports (Grollman & Marcus, 1966; Shen et al., 1968; Jarkovsky et al., 1970). No report has yet appeared concerning $\alpha1\rightarrow3$ fucosyltransferase.

The present paper briefly describes some properties of these three fucosyltransferases, which were purified from pooled samples of human milk from different donors.

The purification method was essentially a two-step procedure. The defatted milk was first applied to an ion-exchange column containing SP (sulphopropyl)-Sephadex C-50; by use of a salt gradient the three fucosyltransferases were separated from each other. In

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