Pyridine Nucleotide-linked Dehydrogenases and Isozymes of Normal Rat Breast and Growing and Regressing Breast Cancers

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Summary

Four pyridine nucleotide-linked dehydrogenases, glucose-6-phosphate dehydrogenase (G6PDH), isocitric dehydrogenase (ICDH), lactic dehydrogenase (LDH), and malic dehydrogenase (MDH), and their isozymes have been studied in the normal virgin rat breast, in the breast of tumor-bearing rats, in the breast cancers themselves, and in these tissues following ovariectomy. Enzyme activity expressed per mg DNA-P facilitates comparison of tumor and normal cells. In breast cancer, the total LDH and G6PDH activity increased, MDH activity decreased, and the proportions of LDH and MDH isozymes were altered. The Kaplan M type of LDH isozyme (2) is more prominent in tumors. Ovariectomy resulted in regression of tumor size accompanied by decrease in total G6PDH and LDH activity but did not restore to normal the proportions of LDH or MDH isozymes.

Introduction

Experimental breast cancers show high rates of glycolysis and increased activity of various enzymes in the aerobic and anaerobic glycolytic pathways. Kaplan (2) has correlated LDH isozyme distributions with metabolic function. We investigated isozymes as well as quantitative enzyme activity of normal and malignant breast tissue and the growing versus regressing tumors.

Materials and Methods

Female Wistar/Furth rats, received at 28 days of age from the A. R. Schmidt Company, Madison, Wisconsin, were housed in light- and temperature-controlled quarters and given Purina rat chow and water ad libitum. Breast cancers were induced by giving intragastrically 10 mg of 3-methylcholanthrene (3-MC) in 1 ml of olive oil 3 times weekly from age 50 to 100 days.

The rats were palpated weekly for tumors, and measurements of tumors were taken in 3 diameters by Jamieson calipers. Following ovariectomy, tumor measurements were made on alternate days to verify regression. Tumors designated as regressing had decreased at least 5 mm in mean diameter by 12 days following ovariectomy. Ovaries and fallopian tubes were removed under ether anesthesia by midline incision in 1 group of animals.

Biochemical determinations were done 12 days after ovariectomy or on paired intact controls. Mammary tissue and tumor were removed under ether anesthesia and kept cold during weighing and preparation of homogenates. Fresh homogenates were used for quantitative determination of enzyme activities and DNA-P and protein measurements and for starch gel electrophoretic separation of the isozymes. Quantitative measurements of G6PDH, ICDH, LDH, and MDH were done by methods previously described for mouse breast (5, 6) and validated for rat breast. Protein was determined by the Lowry method (9) and DNA-P by that of Robins (8).

Starch gel electrophoresis was done by the Smithies technic (12), with 4.7 volts/cm used for 6 hr at 4°C. For LDH and MDH, gels were made up in veronal buffer, ionic strength 0.10, pH 8.5. Gels for ICDH and G6PDH were made with Tris-citrate buffer, ionic strength 0.076 and 0.03 respectively, pH 8.65, and were electrophoresed against borate, ionic strength 1.8, pH 8.2. Following electrophoresis the gels were incubated in the dark for 90 min at 37°C. For all 4 enzymes, the basic reaction mixture was 0.15 m PO₄, pH 7.5, and 75 mg/liter of nitroblue tetrazolium, and 120 mg/liter of phenazine methosulfate. For LDH and MDH, the incubation medium was 270 mg/liter of NAD and 112 mm sodium lactate or 37.5 mm malic acid (adjusted to pH 7.25). For G6PDH and ICDH, NADP was 38 mg/liter, MgCl₂ was 0.5 mm, and glucose-6-phosphate or sodium isocitrate was 2.7 mm.

Results

Table 1 summarizes the quantitative findings. In intact animals, the “normal” breast of tumor-bearing rats does not differ from that of normal controls with respect to pyridine nucleotide dehydrogenase activity or DNA-P and protein content. Ovariectomy produces no detectable biochemical change in either the normal breast or the 3-MC-treated breast. Tumor tissue shows an increase in all 4 dehydrogenases on a wet weight basis. (P < 0.001). However, when activity is calculated per weight DNA-P (Table 2) as a best estimate of enzyme activity/cell, MDH values of tumor are significantly decreased from those of normal breast (P < 0.005). The ICDH of tumor is not significantly increased. Both G6PDH and LDH are approximately 3 times greater in tumor than in normal breast (P < 0.001).

In tumors that regress following ovariectomy, both G6PDH and LDH activities are significantly lower than in growing tumors (P < 0.005 and < 0.05, respectively), but are not reduced to control values (Table 2). Neither ICDH nor MDH values are significantly altered in the regressing tumors.
TABLE 1

ANALYSES OF NORMAL AND MALIGNANT BREAST OF INTACT AND OVARIECTOMIZED RATS

<table>
<thead>
<tr>
<th>TISSUE</th>
<th>MOLES OF PRODUCT/kg WET WEIGHT OF TISSUE/hr</th>
<th>gm/kg WET WEIGHT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G6PDH</td>
<td>ICDH</td>
</tr>
<tr>
<td>Intact rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control, breast</td>
<td>0.053 ± 0.003</td>
<td>0.129 ± 0.013</td>
</tr>
<tr>
<td>3-MC, breast</td>
<td>0.065 ± 0.011</td>
<td>0.130 ± 0.007</td>
</tr>
<tr>
<td>3-MC, tumor</td>
<td>1.100 ± 0.057</td>
<td>1.120 ± 0.049</td>
</tr>
<tr>
<td>Ovariectomized rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control, breast</td>
<td>0.055 ± 0.009</td>
<td>0.154 ± 0.008</td>
</tr>
<tr>
<td>3-MC, breast</td>
<td>0.084 ± 0.013</td>
<td>0.179 ± 0.013</td>
</tr>
<tr>
<td>3-MC, tumor</td>
<td>0.017 ± 0.124</td>
<td>0.900 ± 0.062</td>
</tr>
</tbody>
</table>

* Twenty-four animals were used. Values are mean ± S.E. of groups of 6 animals. Protein, DNA-P, and enzyme analyses were all performed on all homogenates. The abbreviations used are: G6PDH, glucose-6-phosphate dehydrogenase; ICDH, isocitric dehydrogenase; LDH, lactic dehydrogenase; MDH, malic dehydrogenase; DNA-P, deoxyribonucleic acid phosphorus; and 3-MC, 3-methylcholanthrene.

TABLE 2

ENZYME ACTIVITY PER WEIGHT DNA-P IN BREAST, GROWING TUMORS, AND REgressing Tumors

<table>
<thead>
<tr>
<th>TISSUE</th>
<th>MOLES OF PRODUCT/gm DNA-P/hr</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G6PDH</td>
<td>ICDH</td>
<td>LDH</td>
<td>MDH</td>
<td></td>
</tr>
<tr>
<td>Normal breast</td>
<td>0.60 ± 0.05</td>
<td>1.45 ± 0.18</td>
<td>10.7 ± 1.3</td>
<td>41.5 ± 3.7</td>
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<tr>
<td>Growing tumors</td>
<td>2.02 ± 0.07</td>
<td>1.98 ± 0.19</td>
<td>36.2 ± 4.3</td>
<td>23.4 ± 2.4</td>
<td></td>
</tr>
<tr>
<td>Regressing tumors</td>
<td>1.02 ± 0.23</td>
<td>1.45 ± 0.15</td>
<td>22.2 ± 4.0</td>
<td>18.9 ± 1.3</td>
<td></td>
</tr>
</tbody>
</table>

* Values are mean ± S.E. Eighteen animals, 6 in each group, were used. See footnote to Table 1 for definitions of abbreviations.

Chart 1 shows the characteristic electrophoretic distributions of LDH, MDH, G6PDH, and ICDH isozymes of normal breast. This pattern was the same in breast from 12 rats. LDH separates into 5 bands, 4 moving toward the anode, and 1 toward the cathode. The fastest moving one (Band 1) is present in least amount. Bands 2, 3, and 4 are approximately equal in intensity, and Band 5 is somewhat lighter.

Starch gels incubated for MDH activity following electrophoresis show 3 bands, 2 toward the anode and 1 toward the cathode. The fastest migrating band is Band A present in least amount. Bands 2 and 3 are approximately equal in intensity, and Band 4 is somewhat lighter.

Starch gels incubated for MDH activity following electrophoresis show 3 bands, 2 toward the anode and 1 toward the cathode. The fastest migrating band is, however, identical in position to the fastest moving band found in portions of the same gels incubated with substrates for G6PDH and ICDH activities. We refer to this as Band A. It appears without pyridine nucleotides and/or substrates in the reaction mixture, but does require phenazine methosulfate. Band A apparently has electrophoretic properties similar to LDH Band 1. When lactate is omitted from the incubation mixture the band appears, but with less color intensity than with the substrate. With a longer period of electrophoresis (9 hr), Band A and LDH Band 1 separate into 2 distinct bands.

In normal breast, MDH Band 1 moves toward the anode and is darker in color than the cathode-directed MDH Band 2. Ex-
Dehydrogenases and Isozymes of Rat Breast

...including Band A, ICDH of normal breast shows only 1 site of electrophoretic localization, as does G6PDH.

In homogenates of the 3-MC-induced breast cancers, the relative amounts of the various LDH and MDH isozymes are changed. Chart 2 compares the characteristic tumor isozyme patterns of LDH and MDH with those of the normal breast. The faster migrating isozymes of LDH decrease in amount as LDH 4 and 5 increase. There is a reversal of relative amounts of MDH 1 and 2, again with a decrease in the band moving toward the anode and an increase in Band 2. However, the migrations of LDH and MDH isozymes are unaltered, as are those of the single bands of ICDH and G6PDH.

The isozymes of homogenates of breast from tumor-bearing animals migrate in the same way as those of the normal controls, and no difference in relative concentration is found by visual estimation.

Ovariectomy has no effect on the distributions of isozymes in the cancers. Migration distances and relative concentrations of the isozymes are the same in regressing tumor as in growing tumor, even though total LDH and G6PDH decrease in regressing tumors (Table 2). The isozymes of normal breast and of breast tissue of tumor-bearing animals are not altered by ovariectomy.

Discussion

The enzyme profile of pyridine nucleotide-linked dehydrogenases in breast cancer differs from that of resting or lactating breast. Our data are very similar to those of Rees and Huggins (10), obtained from a different strain of rats and by different methods for enzyme determinations. In both the Sprague-Dawley and the Wistar/Furth strains, the 3-MC-induced cancers have higher values for LDH than for MDH. We have also found this true for the transplantable Sarcoma 180 in black C57BL/6 mice. Enzyme activities in Sarcoma 180 in moles of product/kg dry wt/hr are: LDH, 91; MDH, 75; G6PDH, 2.8; and ICDH, 1.8 (unpublished data). In contrast, MDH is higher in resting (Table 1) and lactating rat breast (10).

All 4 pyridine nucleotide dehydrogenases show an increase in cancer when compared with normal breast on a wet weight basis. Because a high proportion of the wet weight of breast is fat, DNA-P and protein were measured. Haddock (4) has shown a constant ratio of DNA-P/nucleus in rat breast throughout lactation and involution. DNA-P/tumor cell is not yet known, but in view of the haploid and diploid nuclei in tumors, it is not likely to be lower than in normal breast cells. When enzyme activity is calculated/weight DNA-P, MDH in the cancers is 0.5 that in normal breast. ICDH is not significantly different. LDH and G6PDH are each approximately tripled in cancer.

These increases of LDH and G6PDH are of functional significance in vivo and in vitro. Aerobic glycolysis of rat mammary tumors in vitro is higher than of lactating breast (10). Glucose administration causes high rates of lactic acid formation (1) and a decrease of pH (13) in mouse mammary cancer tissue in vivo.

These rat-breast cancers not only have increased LDH activity but also have increased proportion of the LDH isozymes that favor lactate formation, the Kaplan M type (2). Lactating rat breast, on the other hand, although high in LDH activity, has the same relative proportions of each isozyme as does involuting (3) or resting breast (Chart 1).

In breast cancer there is a higher proportion of MDH Band 2 than in normal breast. The decrease in MDH activity/weight DNA-P may be due to decreased synthesis of MDH Band 1.

Approximately 90% of these breast cancers regress following ovariectomy, but subsequently regrow. The tumors are various types and mixtures of adenocarcinoma-adenopapillary, fibro-adenopapillary, and occasionally undifferentiated carcinoma. The regressing tumors have the flattened epithelial cells that Huggins et al. (7) and Young et al. (14) have observed in tumors after ovariectomy. However, we observe similar areas in some rapidly growing tumors on unoperated rats. The small proportion of cells altered following ovariectomy seems inadequate to explain the large decrease in LDH and G6PDH activities. The regressing tumors show no change in the isozymes studied. The distributions of LDH and MDH isozymes are the same as in the growing tumors. Total LDH and G6PDH activities decrease toward, but do not achieve, normal levels. MDH decreases are not restored by ovariectomy and regression of tumor.

No detectable change in total enzyme activities or in isozyme distributions were found in normal breast following ovariectomy.

No enzyme alterations were found in the breast of tumor-bearing animals. Similarly, the histologic changes are localized rather than generalized lesions (11).

References

10. Rees, E. D., and Huggins, C. Steroid Influences on Respira-


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