METABOLIC ADAPTATIONS IN RAT HEPATOMAS: ALTERED REGULATION OF SERINE DEHYDRATASE SYNTHESIS BY GLUCOSE AND AMINO ACIDS IN HEPATOCELLULAR CARCINOMAS

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SUMMARY

The forced feeding of amino acid mixtures to rats bearing the Morris 5123 hepatoma had virtually no effect on changing the already high levels of serine dehydratase present in this neoplasm. On the other hand a similar regimen did stimulate an increase in the level of serine dehydratase in the Morris 7800 and Reuber H-35 hepatomas. Glucose administration together with the amino acids had virtually no effect on changing or inhibiting the stimulated levels of the amino acids in the hepatomas. By quantitative precipitin techniques and pulse labeling with valine-14C, it was demonstrated that the rate of synthesis of serine dehydratase in the Morris hepatoma 5123 was extremely high relative to total soluble protein synthesis and that the administration of amino acids or glucose had no effect on valine incorporation into serine dehydratase. The results obtained apparently were not the result of a dramatically different structure of the serine dehydratase of the neoplasms, nor were they a result of the relative unavailability of glucose to the neoplasms because of their different blood supplies. The data presented indicate a deranged effect of glucose on enzyme synthesis, probably the result of altered mechanisms of the translational control of enzyme synthesis.

INTRODUCTION

Earlier studies from this (12, 14, 17) and other laboratories (20) have demonstrated that the hormonal and substrate regulation of enzyme levels in hepatomas is in general altered in comparison with the same mechanisms seen in normal liver. Examples of this have been seen with the regulation of serine dehydratase (L-serine hydro-lyase, EC 4.2.1.13) in the Morris hepatoma 5123 and 7793. The enzyme was shown to be at extremely high levels in tumors growing in intact hosts. Adrenalectomy of the tumor-bearing host resulted in a marked lowering of the levels of serine dehydratase in the tumors but had very little effect on the enzyme in the livers (1). With other enzymes such as tyrosine transaminase the activity was high in most hepatomas and also lowered upon adrenalectomy of the host. Administration of corticosteroids resulted in a marked increase in the level of the enzyme activity in both tumor and liver. The feeding with high-protein diets (5, 13) also failed to stimulate increases in the levels of serine dehydratase in most tumors. An exception to this was the Morris hepatoma 7800 (1), which did respond to a high-protein diet by an increased serine dehydratase level.

In most of the experiments previously described the enzymes studied were those involved in gluconeogenesis. Studies from this laboratory have demonstrated that the administration of glucose to animals being or having been given large amounts of protein results in a complete cessation of the synthesis of the enzyme, serine dehydratase (6, 9, 13). The experiments described in this paper were designed to test whether or not glucose repression of serine dehydratase is active or nonfunctional in several highly differentiated hepatocellular carcinomas.

MATERIALS AND METHODS

The tumors used in these experiments were the Morris 5123 and 7800 hepatomas and Reuber hepatoma H-35. The Morris hepatoma was carried in Buffalo strain rats and the Reuber hepatoma was carried in ACI/N rats. Transplantation of all these neoplasms was performed in this laboratory via trocar intramuscularly in the hind legs of the animals. Induction of serine dehydratase was performed by intraperitoneal injections of an equimolar mixture of 10 essential amino acids at a level of 300 mg in 5 ml 0.9% NaCl solution/dose every 6 hr (8). The glucose, given at the various times indicated or given 6 hr after the initial amino acid administration, was administered through a stomach tube with the mice under slight ether anesthesia. The dose was 2 g glucose/100 g body weight. At the time indicated in each experiment a 14C-labeled amino acid was injected intraperitoneally and the rats were killed after 30 min (6). A high-speed centrifugate of the liver and tumor homogenate was prepared and the 14C-labeled amino acid incorporation into immunochemically precipitable enzyme was determined.
The serine dehydratase antiserum was obtained from rabbits given injections of a highly purified serine dehydratase obtained from rat liver (6). Immunochemical titrations were carried out with an excess and constant amount of antibody incubated with liver or tumor extracts to which cold serine dehydratase of liver had been added to correct for non-specific adsorption of radioactivity (6). After a 24-hr incubation at 3–4°C the antigen-antibody complex was centrifuged out at 2000 rpm and the precipitate was washed 3 times in 0.9% NaCl solution. The precipitate was then dissolved in 0.5 ml 0.5 N sodium hydroxide and the radioactivity of the solution was determined in a Packard scintillation counter. Serine dehydratase activity of liver and tumor was determined with an automated procedure previously described (18).

The 14C-labeled amino acids were obtained from New England Nuclear Corp., Boston, Mass. Both were universally labeled. The specific activity of L-proline-14C was about 80 mCi/mmole. The specific activity of the L-valine-14C was greater than 180 mCi/mmole. All other chemicals used were of the purest grade obtainable.

RESULTS

Immunochemical Reactions of Serine Dehydratase Antigen from Hepatoma 5123 and Normal Rat Liver. Because purification of serine dehydratase from rat tissues has thus far been accomplished only with liver, antibodies to the enzyme prepared in rabbits would be expected to be specific for the rat liver serine dehydratase. In order to carry out quantitative immunochemical reactions with tumor enzyme it became necessary to determine the cross-reactivity of the serine dehydratase from the hepatomas and that of liver. Fig. 1 shows a double diffusion test (7) with antibody to the rat liver enzyme and serine dehydratase preparations from both liver and hepatoma 5123. A single precipitin band with complete coalescence indicates the immunological identity of serine dehydratase from the 2 sources. The quantitative precipitin reaction between hepatoma and liver serine dehydratase and rat liver anti-serine dehydratase antibody is seen in Chart 1. The high-speed supernatant fraction, 0.01 to 0.1 ml, from hepatoma or liver was incubated with 0.2 ml antiserum in a 0.5-ml total volume. Incubation for the antigen-antibody reaction was carried out as described in "Materials and Methods." As seen in Chart 1, the equivalence zone for serine dehydratase antigen from the 2 different tissues was found to be essentially the same. Essentially identical results were obtained with the serine dehydratase antigen of the Reuber H-35 and Morris 7800 hepatomas. As in the case of tryptophan pyrrolase in liver and hepatomas (4), there does not seem to be any significant difference between the antigenic structure of serine dehydratase from normal rat liver and at least 3 highly differentiated hepatocellular carcinomas.

The Effect of Amino Acids and Glucose on Serine Dehydratase Levels in Liver and Hepatoma. With the use of the dietary regimen outlined in "Materials and Methods" the response of serine dehydratase to amino acid administration with and without glucose was studied in the Morris hepatomas 5123 and 7800 and Reuber hepatoma H-35. The results of these experiments are seen in Table 1. Although, as indicated, the response to amino acid administration seen in the host liver was maximal by 12 hr the level of serine dehydratase in the Morris 5123 hepatoma was quite high at 0 time and did not change significantly throughout the remainder of the experiment. As indicated in "Materials and Methods" amino acids with and without glucose were given at 6-hr intervals beginning at 0 time and the animals were sacrificed at the points indicated. In contrast to the 5123 hepatoma the Reuber H-35 hepatoma did show a significant increase in the level of serine dehydratase by 12 hr. In the Morris 7800 hepatoma no significant increase in serine dehydratase occurred until 24 hr after the beginning of amino acid administration. In either case, however, the administration of glucose together with the amino acids did not repress the level of the enzyme but rather in many instances actually increased the enzyme level. This was most notable 6 hr after the initial administration of amino acids and glucose in the Reuber H-35 hepatoma. The only suggested effect of glucose in repressing enzyme levels was seen with the Morris 7800 hepatoma at 24 hr. At this point there was a significantly lower level of serine dehydratase in the hepatoma as compared to the level in hepatomas in animals given amino acids alone. However, glucose had no effect during the first 18 hr of the experiment. There is no circadian rhythm of serine dehydratase in the Morris 7800 hepatoma (19). Therefore, the conclusion from these experi-
Table 1

The effect of amino acids and glucose on serine dehydratase levels in liver and hepatoma

Each value represents the average of 3 to 7 animals. Serine dehydratase was assayed automatically as described previously (15). aa, amino acid mixture outlined in “Materials and Methods.” See text for further details of the experiment. Values for host liver were taken from animals bearing the hepatomas 7800 and H-35.

<table>
<thead>
<tr>
<th></th>
<th>Hepatomas (µmoles product/hr/g ± S.E.)</th>
<th>Host liver (µmoles product/hr/g ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Morris 5123</td>
<td>Morris 7800</td>
</tr>
<tr>
<td>0 time</td>
<td>1800 ± 280</td>
<td>190 ± 20</td>
</tr>
<tr>
<td>6 hr + aa</td>
<td>1950 ± 400</td>
<td>240 ± 80</td>
</tr>
<tr>
<td>6 hr + aa + glucose</td>
<td>2190 ± 600</td>
<td>560 ± 190</td>
</tr>
<tr>
<td>12 hr + aa</td>
<td>2120 ± 200</td>
<td>230 ± 50</td>
</tr>
<tr>
<td>12 hr + glucose</td>
<td>2600 ± 800</td>
<td>390 ± 60</td>
</tr>
<tr>
<td>18 hr + aa</td>
<td>2460 ± 650</td>
<td>217 ± 40</td>
</tr>
<tr>
<td>18 hr + aa + glucose</td>
<td>1800 ± 360</td>
<td>280 ± 40</td>
</tr>
<tr>
<td>24 hr + aa</td>
<td>382 ± 60</td>
<td></td>
</tr>
<tr>
<td>24 hr + aa + glucose</td>
<td>230 ± 40</td>
<td></td>
</tr>
</tbody>
</table>

Table 2

The effect of glucose and amino acids on valine-14C incorporation into serine dehydratase antigen in the Morris hepatoma 5123 and normal liver

All values represent the average of 3 or more rats. The specific activity of the amino acids is given in “Materials and Methods.” L-Valine-14C (20 µCi) was given to the appropriate rats by intraperitoneal injection 40 min prior to sacrifice.

<table>
<thead>
<tr>
<th></th>
<th>Normal liver</th>
<th></th>
<th>Hepatoma 5123</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SDH b units/g</td>
<td>Valine-14C incorporation (dpm/g)</td>
<td>SDH b units/g</td>
<td>Valine-14C incorporation (dpm/g)</td>
</tr>
<tr>
<td>0 time</td>
<td>4.4 ± 0.4</td>
<td>66.0 ± 23.0</td>
<td>320,000</td>
<td>1,880 ± 300</td>
</tr>
<tr>
<td>12 hr + amino acids</td>
<td>125.6 ± 27.1</td>
<td>1,816.0 ± 396.0</td>
<td>325,000</td>
<td>2,500 ± 700</td>
</tr>
<tr>
<td>12 hr + amino acids + glucose</td>
<td>5.6 ± 0.5</td>
<td>40.5 ± 24.3</td>
<td>324,000</td>
<td>2,700 ± 650</td>
</tr>
</tbody>
</table>

bThese values are taken from the data of Jost et al. (6). The animals were actually sacrificed at 10 hr after 0 time.

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Permeability of Liver Host Liver and Morris Hepatomas 5123 and 7800 to Labeled 2-Deoxy-D-glucose-14C

2-Deoxy-D-glucose-14C, 1 μCi/100 g body weight, was injected i.p. into 3 series of rats. In 1 series normal rats were used as a control. In the other 2 series rats bearing the 5123 and the 7800 Morris hepatomas were used. The rats received no glucose at the time of injection. After 20 mm of incorporation the soluble and trichloroacetic acid-insoluble counts were determined. The values given are each the average of 2 animals.

<table>
<thead>
<tr>
<th>Series</th>
<th>Total</th>
<th>Trichloroacetic acid-soluble</th>
<th>Trichloroacetic acid-insoluble</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal liver</td>
<td>29,000</td>
<td>27,000</td>
<td>1,700</td>
</tr>
<tr>
<td>Host liver 7800</td>
<td>31,000</td>
<td>30,000</td>
<td>1,562</td>
</tr>
<tr>
<td>Tumor 7800</td>
<td>31,000</td>
<td>29,000</td>
<td>1,612</td>
</tr>
<tr>
<td>Host liver 5123</td>
<td>35,000</td>
<td>32,000</td>
<td>2,212</td>
</tr>
<tr>
<td>Tumor 5123</td>
<td>29,000</td>
<td>27,000</td>
<td>1,556</td>
</tr>
</tbody>
</table>

Permeability of Liver and Hepatoma to Labeled Glucose Analogs. As previously emphasized (12), because of the different blood supplies of liver and tumor it is of extreme importance to ensure that the regulatory agent being studied is available to both liver and tumor in roughly equivalent amounts. Previous studies have indicated that tryptophan (4) does permeate liver and tumor in equivalent amounts. In order to test this question with respect to glucose, 2 relatively nonmetabolizable glucose analogs which are transported by the same mechanism as glucose have been used. The compounds used were 2-deoxy-D-glucose-14C and α-methyl-D-glucoside (3), universally labeled with 14C. In Tables 3 and 4 are the data obtained with these 2 glucose analogs. In each case 1 or 1.5 μCi of the label were injected i.p. and 15 or 20 min later the animals were killed, the liver and the tumor were homogenized, and denaturable material was precipitated with 10% cold trichloroacetic acid. At this time the label in a sample of blood was essentially negligible and thus any labeled material in a tissue was considered as intracellular. Table 3 shows that the incorporation of...
2-deoxy-D-glucose was essentially the same when normal liver, host liver, and 2 hepatomas were compared. With α-methyl-D-glucoside (Table 4) the incorporation into the acid-soluble fraction of hepatoma 5123 resulted in a slightly lower value than that seen with normal liver. For a closer study of this a time curve (Chart 2) was obtained with normal and host liver as well as with hepatoma 7800. Initially, there was considerably more incorporation into liver than into the hepatoma. After 16 min of incorporation the values for normal liver and the Morris hepatoma 7800 were comparable, whereas the incorporation into the host liver was much higher than either of these tissues. These data strongly indicate that the lack of effect of glucose on the synthesis of serine dehydratase is not the result of the lack of availability of glucose to the neoplastic tissue.

![Fig. 1. Ouchterlony diffusion pattern of serine dehydratase and its antiserum. Wells 1, 2, and 3 contain normal liver extract while Wells 4, 5, and 6 contain extract from Morris hepatoma 5123. The time of immunodiffusion was 24 hr at 4°C. The wells are numbered counterclockwise starting with Well 1.](image)

**DISCUSSION**

Earlier studies from this laboratory indicated that the feeding of a 2% protein diet which contained approximately 80% of its composition in sucrose did not affect the extremely high level of serine dehydratase found in hepatoma 5123 (17). Further studies indicated that the high levels of ornithine-δ-transaminase, an enzyme found in Morris hepatomas 5123 and 7793, was also not affected by the administration of dietary glucose (16). The studies reported herein were designed to extend these experiments by means of the forced feeding regimen previously described (9) and to determine whether or not the rate of serine dehydratase synthesis in at least 3 highly differentiated hepatocellular carcinomas, the Morris 5123 and 7800, and the Reuber H-35 hepatoma. In the Reuber H-35 hepatoma, although amino acid administration significantly raised the level of serine dehydratase, glucose still had no affect in repressing this apparent induction. Utilization of labeled glucose analogs demonstrated that the availability of glucose to the neoplasms was not significantly different from that of liver. On the other hand it is probable that glucose per se is not the primary effector of the glucose regulation of enzyme levels because glucagon administration prevents the glucose effect yet raises blood sugar (9). Recent studies from several laboratories suggest a role of cyclic adenosine monophosphate in the mediation of glucose effects both in microorganisms and in mammalian tissues (11).

Studies reported previously have demonstrated that the synthesis of serine dehydratase in the hepatomas investigated in the experiments reported here varies in its sensitivity to actinomycin D (15). These data have been interpreted as indicating that the cytoplasmic stabilization of the mRNA template for serine dehydratase is different in different tumors. In the studies reported herein it seems that in the Morris hepatoma 5123, in which the synthesis of serine dehydratase is always resistant to actinomycin D, glucose has
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no effect in altering the rate of enzyme synthesis. These data suggest that the mechanism regulating enzyme synthesis at the translational level may be defective in this neoplasm. This is substantiated by the studies (6) which have shown that the repression of serine dehydratase synthesis in rat liver may occur during periods in which enzyme synthesis is completely independent of RNA synthesis. The fact that glucose administration in vivo did little if anything to alter the induced levels of serine dehydratase in the Reuber H-35 and Morris 7800 hepatomas may indicate a loss in translational regulation by glucose in the case of these 2 tumors.

From the studies demonstrating the immunological identity of the hepatoma and liver enzymes, as well as the relatively similar permeability to glucose analogs of the neoplasms and liver, the molecular defects resulting in the failure of glucose repression in these tumors are probably not due to alterations in enzyme structure or to the availability of glucose and amino acids to the tumors. Rather, the results presented herein seem to substantiate the hypothesis that the altered regulation of serine dehydratase synthesis by glucose and amino acids may be the result of defects in mechanisms regulating enzyme synthesis at the translational level (10).

REFERENCES

Metabolic Adaptations in Rat Hepatomas: Altered Regulation of Serine Dehydratase Synthesis by Glucose and Amino Acids in Hepatocellular Carcinomas

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