ABSTRACT

Glutaminase and glutamine synthetase activities in human cirrhotic liver and hepatocellular carcinoma were determined for comparison with normal liver tissues. In hepatocellular carcinoma, glutaminase activity was approximately one-third of that in normal liver, whereas no detectable change in the enzyme activity was observed in cirrhotic liver. Phosphate-dependent and phosphate-independent glutaminase activities were increased approximately 20-fold and 6-fold, respectively, both in the carcinoma and cirrhotic liver compared with those from normal liver. Oxypolarographic tests showed that the rate of glutamine oxidation in the tumor and cirrhotic liver mitochondria was about 5-fold higher than that in the liver mitochondria. The rate of glutamate oxidation in the liver mitochondria was comparable to that in the cirrhotic liver and tumor mitochondria. Glutamine oxidation was inhibited by prior incubation of the mitochondria with 6-diazo-5-oxo-L-norleucine, which inhibited mitochondrial glutaminase. These results indicate that the product of glutamine hydrolysis, glutamate, is catabolized in the tumor and cirrhotic liver mitochondria to supply ATP.

In the liver and cirrhotic liver mitochondria, glutamate was oxidized via the routes of transamination and deamination. On the other hand, glutamate oxidation was initiated preferentially via a transamination pathway in the tumor mitochondria.

RESULTS AND DISCUSSION

Glutamine synthetase and glutaminase activities in hepatocellular carcinomas and cirrhotic liver tissues are presented in Table 1 for comparison with normal liver tissues. The ATP-dependent formation of glutamine from glutamate and ammonia, catalyzed by glutaminase synthetase, plays an important role in normal cellular glutamine metabolism and this enzyme activity is high in the liver. Glutamine synthetase activity was reported to be low in several lines of rat hepatomas (13, 14). Similar results were obtained in a series of hepatoma cells of human and rat origins (6). Our results shown in Table 1 address additional indications that this enzyme activity is markedly decreased in human hepatocellular carcinomas thus far examined. On the other hand, decrease in glutamine synthetase activity was not detectable in cirrhotic liver tissues.

Since prominent glutaminase activities were detectable in both the hepatoma and cirrhotic liver (Table 1), additional experiments on glutamine metabolism in these mitochondria were undertaken. Fig. 1 shows representative oxypolarographic tracings of oxygen consumption by liver and hepatocellular carcinoma mitochondria with either glutamine or glutamate as substrates. Glutamine oxidation was prominent in the tumor mitochondria (Fig. 1c), while mitochondria from the liver showed a feeble glutamine oxidation (Fig. 1a). Both liver and hepatoma mitochondria utilized glutamate well for oxidation (Fig. 1, b, d). Prior incubation of the tumor mitochondria with...
6-diazo-5-oxo-L-norleucine at 0°C for 5 min markedly blocked glutamine oxidation (Fig. 1e) at 5 mM where both glutaminase activities in the mitochondrial homogenates were suppressed. Similar results were obtained with cirrhotic liver mitochondria (data not shown).

Fig. 2 shows the typical representative oxypolarographic tracings of the effects of aminooxyacetate and bromofuroate on the oxygen consumption by liver, cirrhotic liver, and the tumor mitochondria with glutamate as substrate. Oxygen uptake in the liver mitochondria was suppressed either by aminooxyacetate, an inhibitor of glutamate transaminase (15), or bromofuroate, an inhibitor of glutamate dehydrogenase (16) (Fig. 2, a, b). In the cirrhotic liver mitochondria, aminooxyacetate and bromofuroate inhibited the respiration, although the latter had a little inhibitory effect on the cirrhotic liver mitochondria compared with that on the liver mitochondria (Fig. 2, c, d). On the other hand, bromofuroate up to the concentration of 8 mM exerted little or no inhibitory effect on the respiration of the tumor mitochondria, while aminooxyacetate inhibited the respiration (Fig. 2, e, f).

These results indicate that while glutaminase activities were detectable in normal liver, such activities became much higher in the cirrhotic liver and the carcinoma mitochondria, thus allowing them a better oxidation of glutamine. The rate-limiting step in glutamine-dependent oxygen uptake was obviously not glutamine oxidation per se because it was inhibited by 6-diazo-5-oxo-L-norleucine, an inhibitor of glutaminase (17). Consistent with the above interpretation was the finding of little or no glutamine transaminase in these mitochondria. Thus, it was concluded that the product of glutamine hydrolysis, glutamate, was catabolized in the tumor and cirrhotic liver mitochondria to supply ATP. Similar results were obtained with mitochondria from rat hepatoma induced by 3’-methyl-4-dimethylaminoazobenzene. Furthermore, as shown in Fig. 2, in the liver and cirrhotic liver mitochondria, glutamate was oxidized via the routes of transamination and deamination. On the other hand, glutamate oxidation was initiated preferentially via the trans-

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**Table 1 Glutamine synthetase and glutaminase activities**

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Glutamine synthetase (nmol γ-glutamylhydroxamate formed/min/mg protein)</th>
<th>Glutaminase (nmol glutamate formed/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pₐ-dependent</td>
<td>Pᵢ-independent</td>
</tr>
<tr>
<td>Normal human liver (n = 15)</td>
<td>9.80 ± 1.96ᵇ</td>
<td>4.19 ± 0.09</td>
</tr>
<tr>
<td>Cirrhotic liver (n = 11)</td>
<td>7.77 ± 2.18</td>
<td>132.74 ± 60.03</td>
</tr>
<tr>
<td>Hepatocellular carcinoma (n = 11)</td>
<td>3.01 ± 0.71</td>
<td>88.97 ± 52.32</td>
</tr>
<tr>
<td></td>
<td>(P &lt; 0.01)</td>
<td>(P &lt; 0.001)</td>
</tr>
</tbody>
</table>

ᵇ Expressed as subtracted background due to glutamine hydrolysis during incubation without test material.

Statistical analysis was carried out as using Student’s t test.

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**Fig. 1. Oxidative phosphorylation in human liver (a, b) and hepatocellular carcinoma (c–e) mitochondria (Mᵢ) with glutamine (Gln) or glutamate (Glu) as substrate. Incubation conditions were as described in “Materials and Methods.”**

**Fig. 2. Effects of aminooxyacetate (AOA) and bromofuroate (BF) on the oxidation of glutamate (Glu) in human liver (a, b), cirrhotic liver (c, d), and hepatocellular carcinoma (e, f) mitochondria (Mᵢ).**

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2 Unpublished observations.
amination pathway in the tumor mitochondria. These results demonstrate the aberrations in the glutamine metabolism especially in the carcinoma tissues.

The cirrhotic liver still retained the liver function with respect to glutamine synthetase which plays an important role in the liver nitrogen metabolism. Prominent glutamine oxidation and low glutamine synthetase activity observed in the hepatocellular carcinoma tissue may be favorable to the bioenergetics of the tumor cells since the existence of an ATP-dissipating futile cycle due to glutamine synthetase is negligible.

The present findings make us conclude that the imbalance in the glutamine metabolism in the tumor cells is independent of the nature of carcinogenic agent and the species. This conclusion confirms an earlier statement by Prajda (18) that “the biochemical phenotype of liver cancer is independent both from the carcinogen and the species.”

REFERENCES

Glutaminase and Glutamine Synthetase Activities in Human Cirrhotic Liver and Hepatocellular Carcinoma

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