Depression of the Stimulation of Ethanol Oxidation by Fructose or Pyruvate in Liver Cells from Hyperthyroid Animals

MICHAEL N. BERRY and HAROLD V. WERNER

Bruce Lyon Memorial Research Laboratory, Children's Hospital Medical Center, Oakland, Calif. 94609, U.S.A., and Clinical Investigation Center, Oak Knoll Naval Hospital, Oakland, Calif. 94627, U.S.A.

Previous studies have demonstrated that the oxidation of ethanol by morphologically intact isolated cells from rat liver is increased in the presence of fructose (Berry, 1971a). Since hepatic oxidations are generally stimulated in the hyperthyroid state, it was decided to determine whether fructose-stimulated ethanol oxidation would be further enhanced in liver cells from thyroxine-treated animals. Isolated liver cells were prepared in high yield by the method of Berry & Friend (1969) from two groups of Sprague-Dawley rats (weighing 300-350g) deprived of food for 18h to deplete hepatic glycogen. One group received no treatment and were used as controls. The other group received L-thyroxine (60μg/kg body wt. per day) administered intraperitoneally for 1-16 days. All treated animals maintained weight on this regimen. The liver cells were washed three times and incubated in a balanced saline medium (Berry & Kun, 1972). O2 uptake was measured manometrically and metabolites were determined by specific enzymic techniques. Utilization of ethanol was also assessed by measuring the accumulation of [14C]acetate and other 14C-labelled carboxylic acids from [14C]ethanol.

The results in Table 1 show that the basal rate of ethanol oxidation was the same in cells from normal and hyperthyroid animals. However, ethanol oxidation in the presence of fructose was stimulated by only 66% in cells from thyroxine-treated rats compared with a stimulation of 185% in cells from control animals, even though the rate of hepatic O2 uptake was substantially greater in the treated group. Associated with this depression of fructose-stimulated ethanol oxidation in the hyperthyroid state was a marked decrease in the accumulation of reduced products of fructose metabolism, namely sorbitol, glycerol and glycerol 3-phosphate (Table 1). However, gluconeogenesis from fructose was unchanged and lactate formation was increased.

The depression of fructose-stimulated ethanol oxidation by thyroxine treatment appears paradoxical, especially in view of recent findings that oxidation of glycerol and sorbitol by liver cells from hyperthyroid animals is increased by 50-100% (H. V. Werner & M. N. Berry, unpublished work). However, we have developed a model that accounts for the effects of fructose on hepatic ethanol metabolism and provides an explanation for the actions of thyroxine.

In the normal animal the hepatic interactions of fructose and its metabolites with ethanol may involve a number of coupled cytoplasmic redox reactions:

\[
\text{Fructose} + \text{ethanol} \rightarrow \text{sorbitol} + \text{acetaldehyde} \quad (1)
\]

\[
\text{Dihydroxyacetone phosphate} + \text{ethanol} \rightarrow \text{glycerol 3-phosphate} + \text{acetaldehyde} \quad (2)
\]

\[
\text{Glyceraldehyde} + \text{ethanol} \rightarrow \text{glycerol} + \text{acetaldehyde} \quad (3)
\]

It is probable that much of the acetaldehyde formed is oxidized to acetate by an intramitochondrial pathway (Marjanen, 1972). Glycerol arising in reaction (3) may be phosphorylated:

\[
\text{Glycerol} + \text{ATP} \rightarrow \text{glycerol 3-phosphate} + \text{ADP} \quad (4)
\]

and the glycerol 3-phosphate generated in this step or in reaction (2) may then be oxidized in the mitochondria in a reaction catalysed by the flavin- (Fp-)linked glycerol phosphate dehydrogenase:

\[
\text{Glycerol 3-phosphate} + \text{Fp} \rightarrow \text{dihydroxyacetone phosphate} + \text{FpH2} \quad (5)
\]

Hence it seems likely that fructose and its metabolites promote ethanol oxidation by acting as cytoplasmic hydrogen acceptors and by providing carrier molecules for the
Table 1. Interactions of fructose and ethanol in liver cells from normal and hyperthyroid rats

Isolated cells from the livers of rats deprived of food for 18 h were incubated for 40 min at 37°C in a saline medium containing (final concentrations) 140 mM-NaCl, 5.4 mM-KCl, 0.8 mM-MgSO₄, 1 mM-CaCl₂ and 10 mM-sodium phosphate buffer, pH 7.4. Fructose (initial concentration 10 mM) and ethanol (7.5 mM) were present in each flask. Values that are statistically significantly different from normal (P < 0.001) are indicated by *. The basal ethanol oxidation (fructose absent) was 0.76 ± 0.04 μmol/min per g wet wt. for the control group and 0.73 ± 0.03 μmol/min per g wet wt. for liver cells from thyroxine-treated animals. Results are given as means ± S.E.M.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of expts.</th>
<th>O₂</th>
<th>Glucose</th>
<th>Fructose</th>
<th>Lactate</th>
<th>Glycerol</th>
<th>3-phosphate</th>
<th>Sorbitol</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>17</td>
<td>-2.86 ± 0.05</td>
<td>2.62 ± 0.09</td>
<td>-4.19 ± 0.12</td>
<td>0.73 ± 0.04</td>
<td>0.32 ± 0.03</td>
<td>0.19 ± 0.02</td>
<td>0.87 ± 0.04</td>
<td>-2.16 ± 0.09</td>
</tr>
<tr>
<td>Thyroxine</td>
<td>10</td>
<td>-3.97 ± 0.10*</td>
<td>2.81 ± 0.13</td>
<td>-3.96 ± 0.20</td>
<td>1.23 ± 0.07*</td>
<td>0.027 ± 0.006*</td>
<td>0.021 ± 0.004*</td>
<td>0.14 ± 0.03*</td>
<td>-1.21 ± 0.08*</td>
</tr>
</tbody>
</table>
Table 2. Interactions of pyruvate and ethanol in liver cells from normal and hyperthyroid rats

Conditions were as given in Table 1. The initial concentrations of added substrate were 10 or 15 mM for pyruvate and 7.5 mM for ethanol. Pyruvate was present in all vessels and ethanol was added as indicated. Results are given as means ± S.E.M.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of expts.</th>
<th>Ethanol present</th>
<th>Lactate formed (μmol/min per g wet wt.)</th>
<th>Extra lactate formed in the presence of ethanol (μmol/min per g wet wt.)</th>
<th>Ethanol removed (μmol/min per g wet wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>8</td>
<td>−</td>
<td>1.33 ± 0.04</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>None</td>
<td>8</td>
<td>+</td>
<td>3.92 ± 0.23</td>
<td>2.59</td>
<td>2.75 ± 0.21</td>
</tr>
<tr>
<td>Thyroxine</td>
<td>5</td>
<td>−</td>
<td>1.92 ± 0.13</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Thyroxine</td>
<td>5</td>
<td>+</td>
<td>2.98 ± 0.16</td>
<td>1.06</td>
<td>1.39 ± 0.13</td>
</tr>
</tbody>
</table>

operation of the glycerol phosphate shuttle (Zebe et al., 1959): reactions (2) and (5) or reactions (3), (4) and (5). We have recently found this shuttle to be of major significance for the oxidation of cytoplasmic reducing equivalents in rat liver (M. N. Berry, E. Kun & H. V. Werner, unpublished work; see also Berry & Kun, 1972).

We suggest that in the hyperthyroid state the mitochondrial limb of the glycerol phosphate shuttle is greatly stimulated (Lee & Lardy, 1965), but that the cytoplasmic coupled reactions of ethanol with fructose and its metabolites are markedly depressed. In consequence reduced products of fructose metabolism do not accumulate (Table 1) and the entry of ethanol hydrogen into the mitochondria by way of the glycerol phosphate shuttle is impaired.

It is unlikely that these effects of thyroxine treatment are due to a direct inhibitory effect of thyroxine on the NAD-linked dehydrogenases involved in the various cytoplasmic redox reactions, since the hormone is ineffective when added to liver cell suspensions in vitro. Moreover, the decline in fructose-stimulated ethanol oxidation becomes manifest only after the rats have been treated with thyroxine for a period of 2–4 days, during which time the inhibitory effects of ethanol on gluconeogenesis from sorbitol or glycerol are also gradually lost (H. V. Werner & M. N. Berry, unpublished work).

Support for the suggestion that the involvement of ethanol in certain cytoplasmic redox reactions is depressed in the hyperthyroid state comes from study of the interactions of pyruvate with ethanol in cells from thyroxine-treated animals. Pyruvate stimulates ethanol oxidation by liver cells from euthyroid rats and this stimulation is accompanied by the accumulation of large quantities of lactate (Berry, 1971b; and Table 2). In the hyperthyroid state the stimulation of hepatic ethanol oxidation by pyruvate is depressed and there is a corresponding diminution in the rate of lactate accumulation (Table 2). This implies that the cytoplasmic redox reaction between pyruvate and ethanol, mediated by lactate dehydrogenase and alcohol dehydrogenase, takes place less readily in cells from hyperthyroid animals.

Our results cast some doubt on the concept that there is only one functional pool of cytoplasmic NAD (Williamson et al., 1967), since it appears that in the hyperthyroid state cytoplasmic NADH arising from sorbitol oxidation is readily transferred to the mitochondria by means of the glycerol phosphate shuttle, whereas the entry of NADH generated in ethanol oxidation is impaired. In view of these findings the results of studies of the effects of thyroxine on the 'redox state' of the cytoplasmic compartment of the hepatic cell (see, e.g., Hillbom & Lindros, 1971) may require re-evaluation.

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Berry, M. N. (1971a) Biochem. J. 123, 40p
Berry, M. N. (1971b) Biochem. J. 123, 41p

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The Effects of Ethanol on the Activity of Rat Liver Tryptophan Pyrrolase

ABDULLA A.-B. BADAWY and MYRDDIN EVANS

Addiction Unit, Whitchurch Hospital, Cardiff CF4 7XB, U.K.

The activity of haem-dependent tryptophan pyrrolase (L-tryptophan-O2 oxidoreductase, EC 1.13.1.12) is enhanced in livers of rats treated with the porphyrin 2-allyl-2-isopropylacetamide (Feigelson & Greengard, 1961), and patients with porphyria exhibit increased urinary excretion of tryptophan metabolites (Price, 1961). Ethanol produces

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Fig. 1. Effects of ethanol on rat liver tryptophan pyrrolase activity in vivo

Ethanol (5 ml/kg) was injected at zero time. Each point represents the mean value for four rats except that the zero-time points are means for 20 animals. The enzyme activity was measured as described in the text. O, Total enzyme activity (that measured in the presence of 2μM-haematin); ●, holoenzyme activity (that in the absence of added cofactor).