The purpose of this study was to determine whether catalase-dependent alcohol metabolism is activated by alcohol (i.e., swift increase in alcohol metabolism). When ethanol or the selective substrate for catalase, methanol, was given (5.0 g/kg) in vivo 2 to 3 h before liver perfusion, methanol and oxygen metabolism were increased significantly. This increase was blocked when the specific Kupffer cell toxicant GdCl₃ was administered 24 h before perfusion. These data support the hypothesis that catalase-dependent alcohol metabolism is activated by acute alcohol and that Kupffer cells are involved. Ethanol treatment in vivo increased ketogenesis from endogenous fatty acids nearly 3-fold and increased plasma triglycerides and hepatic acyl CoA synthetase activity; all increases were blocked by GdCl₃. These findings support the hypothesis that ethanol increases H₂O₂ supply for catalase-dependent alcohol metabolism by increasing fatty acid supply. Infusion of oleate stimulated oxygen uptake 1.5-fold and methanol metabolism 4-fold, but these parameters were not altered by GdCl₃. Moreover, the effects of ethanol treatment were blocked by the cyclooxygenase inhibitor indomethacin, and prostaglandin E₂ (PGE₂) was increased more than 200% in media from cultured Kupffer cells from rats treated with ethanol in vivo. Furthermore, lipoprotein lipase activity in retropitoneal fat pads, which is known to be inhibited by PGE₂, was reduced 70% by ethanol. These data are consistent with the hypothesis that Kupffer cells play a key role in activation of catalase-dependent alcohol metabolism, most likely by producing mediators (e.g., PGE₂) that inhibit lipoprotein lipase, increase the supply of fatty acids to the liver, and increase generation of H₂O₂ via peroxisomal β-oxidation.

Based on sensitivity to the alcohol dehydrogenase (ADH) inhibitor 4-methylpyrazole, it was reported previously that ADH was involved in the swift increase in alcohol metabolism (SIAM) (Yuki and Thurman, 1980). This phenomenon is characterized by increased basal oxygen uptake, which provides NAD⁺ for ADH-dependent alcohol metabolism (Yuki and Thurman, 1980). As glycogen reserves are depleted, glycogenolysis is slowed and ADP is shuttled into the mitochondria, where respiration is increased. More recently, however, it has been shown that 4-methylpyrazole also inhibits acyl CoA synthetase (Bradford et al., 1993a), a pivotal enzyme in the synthesis of acyl CoA compounds required for the generation of H₂O₂ via peroxisomal β-oxidation. Catalase is localized in the peroxisome, and the catalase pathway requires H₂O₂, which is provided largely by metabolism of fatty acids via peroxisomal β-oxidation (Lazarow and de Duve, 1976; Handler and Thurman, 1988). Methanol is a known selective substrate for catalase in rodents and is an excellent tool for the evaluation of catalase-dependent alcohol metabolism without the use of inhibitors (Feytmans et al., 1974; Bradford et al., 1993a). It is well known that ethanol stimulates peripheral lipolysis and increases circulating triglycerides (Khanna et al., 1974); however, whether catalase participates in the mechanism of SIAM is not known.

It has been demonstrated that Kupffer cells, the resident hepatic macrophages, participate in the pathophysiology of ethanol-induced liver damage (Adachi et al., 1995). When Kupffer cells are activated, potent cytokines such as platelet-activating factor and tumor necrosis factor-α are released. In addition, Kupffer cells release prostaglandin E₂ (PGE₂), which stimulates oxygen uptake by parenchymal cells via increases in cAMP (Qu et al., 1996). GdCl₃ specifically destroys large Kupffer cells without causing other morphological changes in liver (Hardonk et al., 1992). Moreover, GdCl₃ diminishes inflammation and necrosis due to ethanol (Adachi et al., 1994) and fibrosis using model compounds (Sullivan et al., 1995; Wall et al., 1995). Furthermore, GdCl₃ treatment blocked the increase in hypoxia and production of α-hydroxyethyl radicals associated with chronic ethanol exposure.
Materials and Methods

Treatment of Rats. Fed, female Sprague-Dawley rats (100–120 g) were used in this study. GdCl₃ (10 mg/kg) dissolved in acidic saline (pH 3.0) was injected into the tail vein 24 h before perfusion in some rats. This dose of GdCl₃ has been shown to remove large Kupffer cells without causing other morphological changes in the liver (Hardonk et al., 1992). Moreover, it eliminates about 80% of Kupffer cells based on mRNA for a specific Kupffer cell lectin (Koop et al., 1997). Ethanol (5.0 g/kg), methanol (5.0 g/kg), and olive oil (2 ml/100 g b.wt.), a good source of oleate, were administered intragastrically 2.5 h before liver perfusion. In some experiments, indomethacin (3.0 mg/kg, in dimethylsulfoxide) was administered intragastrically 1 h before ethanol.

Liver Perfusion and Alcohol Metabolism. Livers were perfused using hemoglobin-free Krebs-Henseleit buffer under conditions that were established more than 30 years ago (Scholz, 1968) and have been used for studies of hepatic metabolism, oxidation of xenobiotics, and metabolism of alcohols (reviewed in Brouwer and Thurman, 1996). The oxygen concentration in the effluent perfusate was monitored using a Teflon-shielded, Clark-type oxygen electrode. After oxygen uptake reached steady state values in about 15 min, the perfusion system was converted to a closed system with a 50-ml volume containing either 25 mM ethanol or methanol. Perfusion was reoxygenated using a Silastic tube oxygenator (Handler et al., 1986). Samples of perfusate (0.5 ml) were collected every 10 to 15 min, and the decrease in alcohol concentration over time was measured with head-space gas chromatography as described in detail elsewhere (Bradford et al., 1993a). Rates of alcohol metabolism were calculated based on changes in concentration over time and were expressed per gram of liver per hour. After perfusion, the liver was fixed with a solution of 5% buffered formalin and was perfused with alcohol for 15 min. Alcohol elimination was monitored during this period to correct for vaporization of alcohol from the organ surface, which was minimal.

Enzyme Measurements. Liver homogenates (1:10) were prepared in 0.25 M sucrose, and activities of catalase, acyl CoA synthetase, and acyl CoA oxidase were determined as described previously (Bradford et al., 1993a). Lipoprotein lipase (LPL) activity was determined in homogenized retroperitoneal fat pads as described elsewhere (Borensztajn et al., 1970). Measurement of LPL activity required chylomicrons that were harvested from fasted rats treated for 4 h with olive oil (2 ml/100 g b.wt. i.g.). Rats were anesthetized with pentobarbital (50 mg/kg) and chylomicrons (100–150 μEq of triglyceride fatty acid/ml) were collected from the thoracic duct for 2 h and frozen (~20°C) for subsequent use (Borensztajn et al., 1970). Rats were anesthetized, and retroperitoneal fat pads were harvested and homogenized in saline (1:40). Homogenates (56 μl) were incubated at 37°C for 2 h in 0.1 ml of a cocktail containing 2 volumes of albumin (20% w/v in water, pH 8.1), 1 volume of 0.7 M Tris-HCl (pH 8.1), and 0.5 volume each of serum, heparin (14 IU/ml), and chylomicrons to provide appropriate substrates for LPL. The reaction was stopped by adding 50 μl of incubation mixture to 250 μl of Dole's extraction mixture, and free fatty acids were extracted. Free fatty acids were washed with heptane, isolated, and determined colorimetrically (Novak, 1965). Triglycerides were determined in plasma using a spectrophotometric assay after hydrolysis to glycerol and free fatty acids (Bucolo and David, 1973).

PGE₂ from Kupffer Cells. Rats were treated with saline or ethanol (5.0 g/kg) and were killed 2 h later for isolation of Kupffer cells. Isolation was performed using collagenase digestion and differential centrifugation with Percoll as described previously (Pertoft and Smedsroed, 1987). Primary cultures of Kupffer cells from control or ethanol treated rats were incubated for 4 h. Supernatants were assayed for PGE₂ by competitive radioimmunoassay using 125I-labeled PGE₂ (Advanced Magnetics, Cambridge, MA).

Statistics. Statistical comparisons were made using analysis of variance (ANOVA) with Bonferroni's post hoc comparisons. Student's t test, or two-way ANOVA with Tukey's post hoc comparisons on ranks as appropriate. p < .05 was selected before the study as the level of significance.

Results

Liver Perfusion and Alcohol Metabolism by Perfused Liver after Acute Exposure to Alcohol In Vivo (SIAM). Figure 1 (top) depicts typical liver perfusion experiments from a control animal and a rat 2.5 h after treatment with 5.0 g/kg methanol in vivo. In this experiment, basal rates of oxygen uptake, which were monitored continuously, were around 100 μmol/g/h and were increased to 200 μmol/g/h after methanol treatment in vivo. Values increased about...
20% after the addition of 25 mM methanol to the perfusate, most likely due to an increase in peroxisomal oxygen demand (see also Fig. 3, top). The methanol concentration in the perfusate decreased over time at a rate of 23 μmol/g/h in the control and 67 μmol/g/h after methanol treatment (Fig. 1, bottom). Figure 3 summarizes the effect of methanol treatment in vivo on rates of oxygen and methanol uptake by the perfused liver. Average rates of oxygen uptake by the perfused liver were increased significantly from 114 ± 12 to 192 ± 5 μmol/g/h by methanol treatment in vivo. Methanol uptake was likewise increased from 22 ± 6 to 83 ± 18 μmol/g/h. Thus, like ethanol, methanol, which is not a substrate for ADH in rodents, can produce a SIAM phenomenon.

Figure 2 summarizes the effect of GdCl₃ treatment and ethanol administration in vivo on oxygen uptake (top) and methanol metabolism (bottom) by the perfused liver. Basal rates of oxygen uptake were nearly doubled after treatment with ethanol 2.5 h before perfusion as expected: the “SIAM” phenomenon (Yuki and Thurman, 1980; Bradford et al., 1993b). Treatment with GdCl₃ did not alter basal rates of oxygen uptake, but the increase observed with ethanol treatment was blocked. Rates of methanol metabolism by the perfused liver increased from 22 ± 6 to 55 ± 10 μmol/g/h as a result of 2 to 3 h of ethanol treatment in vivo. It has been demonstrated that when Kupffer cells were destroyed with GdCl₃, the hypermetabolic state due to ethanol treatment in vivo was blocked (Bradford et al., 1993b). In the current study, GdCl₃ treatment also blocked the increase in methanol metabolism due to alcohol treatment (Fig. 2, bottom). Furthermore, the cyclooxygenase inhibitor indomethacin completely blocked the stimulation of oxygen uptake by ethanol. In these experiments, stimulated rates of oxygen uptake (189 ± 18 μmol/g/h) due to ethanol were blunted by indomethacin (119 ± 15 μmol/g/h, p < .03), supporting the hypothesis that eicosanoids are involved in the stimulation of hepatic oxygen uptake after alcohol administration. Primary cultures of Kupffer cells were isolated 2 h after saline or ethanol treatment in vivo, and PGE₂ levels were increased significantly from 47 ± 6 (control) to 144 ± 31 (ethanol; p < .006) pmol/10⁶ cells/4 h.

Effect of Acute Alcohol Treatment on Ketogenesis. Because free fatty acids could provide substrate for catalase-dependent alcohol metabolism, rates of ketogenesis were calculated from ketone body release into the effluent perfusate. Basal rates of ketogenesis were low and not different in those livers from control or GdCl₃-treated rats (Table 1); however, there was a significant, nearly 2-fold increase after acute ethanol treatment in this study, confirming earlier work (Yuki and Thurman, 1980). Moreover, this increase in ketone body production due to ethanol was blocked by GdCl₃ treatment (Table 1). Because peroxisomal fatty acid metabolism generates H₂O₂ for metabolism of alcohols via catalase, it is possible that GdCl₃ blocks the production of H₂O₂ when excess fatty acid is present. To test this hypothesis, rats were given olive oil, a good source of oleate, to provide exogenous fatty acids, a phenomenon that was also not affected by GdCl₃.

Fatty Acid Supply. Because peroxisomal β-oxidation requires fatty acids, plasma triglycerides and several key enzymes involved in lipid metabolism (e.g., LPL and acyl CoA synthetase) were measured. LPL activity in retroperitoneal fat pads was decreased significantly by about 60% after alcohol treatment, an effect blocked by GdCl₃ (Table 1). Plasma triglycerides were increased nearly 2-fold by ethanol treatment as expected, an effect also blocked by treatment with GdCl₃ (Table 2). Hepatic acyl CoA synthetase activity was increased slightly but significantly 2.5 h after ethanol treatment in vivo by 31 to 156 μmol/g/h, a phenomenon that was unaffected by GdCl₃. Methanol metabolism was also increased about 3-fold by the addition of exogenous fatty acids, a phenomenon that was also not affected by GdCl₃.

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**Discussion**

**The Catalase Pathway Is Activated Rapidly by Both Ethanol and Methanol.** When ethanol or methanol was given in vivo, rates of methanol metabolism increased significantly in only a few hours, leading to the conclusion that the catalase pathway is stimulated by alcohols (Figs. 2 and 3).

It has been demonstrated that catalase-dependent alcohol metabolism is regulated by H₂O₂ supply (Oshino et al., 1973), which arises predominately from fatty acid oxidation by peroxisomes (Fig. 4) (Handler and Thurman, 1985). Rates of methanol metabolism were stimulated after treatment with oleate, and values were not altered by the destruction of Kupffer cells, suggesting that these cells do not effect transport of CoA compounds into the peroxisome. Additionally, catalase and acyl CoA oxidase were unchanged by destruction of Kupffer cells with GdCl₃ (see Results). On the other hand, acute ethanol treatment in vivo elevates triglycerides in plasma (Table 2 and Results) due partly to stimulation of lipases by adrenergic hormones and a decrease in retroperitoneal LPL activity (Table 2) (Brodie et al., 1961; Elko et al., 1961). Moreover, increased rates of ketogenesis demonstrate that utilization of fatty acids after alcohol is elevated (Table 1).

In a recent study, it was demonstrated that an acute dose of methanol in vivo significantly elevated fatty acid methyl ester levels in liver (Kaphalia et al., 1995) and increased levels of palmitic, stearic, linoleic, oleic, and arachidonic acids within 3 h. When methanol was given 2.5 h before perfusion here, rates of oxygen uptake and methanol metabolism were stimulated significantly, consistent with the hypothesis that increased levels of long-chain fatty acids provide substrate for production of H₂O₂ necessary for catalase-dependent alcohol metabolism (Fig. 3). It has previously been demonstrated that rates of methanol metabolism by the treatment (Table 2), and this effect was also blocked by GdCl₃. On the other hand, catalase was unaltered by GdCl₃ (control, 2891 ± 367 U/g liver; GdCl₃, 2781 ± 180 U/g liver), and catalase and acyl CoA oxidase remained unchanged (control, 0.8 ± 0.3 nmol/mg protein/min; GdCl₃, 1.1 ± 0.6 nmol/mg protein/min).

**Table 2**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plasma Triglycerides (mg/dl)</th>
<th>Fat Pad LPL (U/g tissue)</th>
<th>Liver Acyl CoA Synthetase (nmol/mg protein/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>19.1 ± 1.6</td>
<td>76.0 ± 14.9</td>
<td>14.1 ± 1.0</td>
</tr>
<tr>
<td>Ethanol</td>
<td>37.4 ± 7.2</td>
<td>23.7 ± 8.5</td>
<td>17.9 ± 0.7</td>
</tr>
<tr>
<td>GdCl₃ + ethanol</td>
<td>21.8 ± 4.2</td>
<td>52.5 ± 21.7</td>
<td>16.4 ± 1.2</td>
</tr>
</tbody>
</table>

* p < .05 for comparison with the control group.
perfused liver can be elevated significantly when fatty acids are infused (Handler and Thurman, 1987). Here, catalase-dependent methanol metabolism in perfused liver was increased from 16 to 67 μmol/g/h by oleate, values that were inhibited completely with the catalase inhibitor aminotriazole (Handler and Thurman, 1987). Under these conditions, ethanol metabolism was diminished about 70%. When aminotriazole was given before ethanol or methanol in deer mice lacking ADH, rates of ethanol and methanol metabolism were diminished nearly completely in vivo (Bradford et al., 1993c). Thus, aminotriazole effectively inhibits catalase-dependent methanol and ethanol metabolism in vivo and in perfused liver. In this study, when oleate was administered alone, providing an excess of fatty acid, oxygen and methanol metabolisms were both stimulated dramatically (Fig. 3). It is concluded that this phenomenon is Kupffer cell independent because it was GdCl₃ insensitive (Fig. 3). Taken together, it is concluded that Kupffer cells participate in regulation of H₂O₂ supply via increasing delivery of lipid to peroxisomal β-oxidation in the liver (see below).

**PGE₂ Participates in SIAM by Providing Lipid.** SIAM requires activation of oxygen uptake and cofactor supply for alcohol metabolism. Is there evidence to support the hypothesis that PGE₂ plays a role in SIAM? A link between Kupffer cells and regulation of oxygen uptake was made recently. Qu et al. (1996) demonstrated that Kupffer cells produce PGE₂ in sufficient quantities to stimulate respiration of isolated parenchymal cells. Media from cultured Kupffer cells isolated from rats fed ethanol chronically stimulated respiration about 30% in parenchymal cells. In this study, PGE₂ was significantly higher in media from Kupffer cells isolated from rats after acute ethanol treatment (see Results). Furthermore, the stimulation of oxygen uptake was blocked by the cyclooxygenase inhibitor indomethacin (see Results) (Qu et al., 1996). This study demonstrated that activation of oxygen uptake in the perfused liver by alcohol is dependent on mediators such as PGE₂ from Kupffer cells.

Several studies have examined the interactions between prostaglandins and fatty acid supply (Feingold et al., 1992; Hardardottir et al., 1992; Flisiak et al., 1993). Regulation of lipolysis has been linked to prostaglandin synthesis (Feingold et al., 1992), and a recent study demonstrated that LPL gene expression in peritoneal macrophages was inhibited by PGE₂ (Desanctis et al., 1994). Additionally, indomethacin was shown to overcome the effects of endotoxin on LPL inhibition (Desanctis et al., 1994) and blocked the increase in PGE₂ due to ethanol in stellate cells (Flisiak et al., 1993). In this study, ethanol stimulated PGE₂ release from Kupffer cells and decreased LPL activity (Table 2). This causes an increase in free fatty acids, which are required substrates for catalase-dependent alcohol metabolism. This phenomenon was blocked when Kupffer cells were destroyed with GdCl₃ as well as ethanol-induced changes in plasma triglycerides and acyl CoA synthetase (Table 2). Thus, it is concluded that PGE₂ from the Kupffer cell plays a pivotal role in the supply of free fatty acids to the liver (Fig. 4).

Taken together, these data clearly support the hypothesis that Kupffer cells are involved in SIAM. The scheme depicted in Fig. 4 summarizes the key elements of this working hypothesis. It is hypothesized that alcohol increases plasma endotoxin (Enomoto et al., 1998), which activates Kupffer cells to produce mediators such as PGE₂ that inhibit peripheral LPL, resulting in an increase in free fatty acids and activation of mitochondrial respiration via cAMP (Hassid, 1986; Garcia et al., 1997). Adrenergic stimulation by alcohol also contributes to increased ketone body formation and triglycerides in the blood. Fatty acyl CoA oxidase generates H₂O₂ for catalase-dependent alcohol metabolism from free fatty acids in the peroxisome. Importantly, ethanol-induced increases in LPL activity, acyl CoA synthetase activity, ketone body formation, and plasma triglycerides returned to normal levels when Kupffer cells were destroyed.

**References**


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