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ETHANOL-INDUCED DISTURBANCE OF HEPATIC MICROCIRCULATION AND HEPATIC HYPOXIA

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Abstract—The hypothesis was tested whether ingestion of ethanol might disturb the hepatic microcirculation with resulting hepatic hypoxia. Infusion of ethanol increased the portal pressure concentration-dependently in rat livers perfused with Krebs–Henseleit buffer at a constant flow rate (E_{max} = 11.5 cm H_2O, EC_{50} = 90 mM). This increase in portal pressure was due to hepatic vasoconstriction, since it diminished in the presence of sodium nitroprusside, a direct acting vasodilator. The regional hepatic tissue hemoglobin concentration after perfusion with added erythrocyte suspension (hematocrit 1%), measured by tissue-reflectance spectrophotometry, was significantly diminished by the infusion of ethanol, indicating the impairment of the microcirculation of the superficial layer of the liver. When the absorption spectrum of the liver was examined by reflectance spectrophotometry, infusion of ethanol caused a parallel reduction of all the mitochondrial respiratory cytochromes in a concentration-dependent fashion, concomitant with the increase of portal pressure, indicating a marked reduction of oxygen concentration in superficial liver tissue. The reduction of the respiratory ctyochromes was also associated with the decrease in oxygen consumption of the liver, indicating that the hepatic hypoxia was due to the reduction of oxygen delivery to hepatocytes rather than the increased oxygen consumption of the liver. The reduction of the respiratory cytochromes was correlated with the increase in portal pressure and was inhibited by sodium nitroprusside. These data indicate that the ethanol-induced hepatic vasoconstriction disturbs hepatic microcirculation, resulting in hepatic hypoxia and reduction of mitochondrial respiratory cytochromes.

The mechanism of the hepatotoxic effects of ethanol is still obscure. Alcoholic liver injury predominates in the pericentral region [1], in which oxygen tension is physiologically lowest [2]. The enhanced injurious effect of ethanol at this site is postulated to be due to hypoxia, resulting from an enhanced oxygen demand of hepatocytes for the oxidative metabolism of ethanol [3, 4]. Since hypoxic episodes induced central necrosis in rat livers treated chronically with ethanol [3, 5], a reduction in the hepatic availability of oxygen, such as respiratory disease, anemia and a decrease in hepatic blood flow, could explain the production of the centrilobular liver injury under these conditions [6, 7]. However, it has been considered that the increased hepatic blood flow following ethanol administration could offset the effect of increased hepatic oxygen consumption by ethanol, since the oxygen tension and the oxygen saturation of hemoglobin in the hepatic venous blood did not decrease after administration of ethanol [8]. Lieber and Sato [9] recently reported that ethanol administration at high dose decreased hepatic oxygen utilization in baboons fed alcohol chronically without the reduction of hepatic vein oxygenation. In their study, the index of regional hepatic tissue hemoglobin concentration assessed by reflectance spectrophotometry [10] showed a decrease after the load of high ethanol dose, suggesting that the ethanol administration disturbed the circulation of the superficial layer of the liver. If hepatic circulation is disturbed by ethanol, focal hypoxia may be produced in the liver without lowering of hepatic vein oxygenation, resulting in an impairment of hepatic oxygen utilization. It has been reported that intravenous administration of ethanol increased hepatic vascular resistance in experimental animals *in vivo* [11, 12] and that ethanol had a vasoconstrictive effect on canine hepatic portal vein *in vitro* [13]. These suggest that ethanol may induce hepatic vasoconstriction. Therefore, we hypothesized that the ethanol might disturb hepatic microcirculation via hepatic vasoconstriction, leading to the focal hepatic hypoxia. In this study, effects of ethanol on hepatic vasculature, hepatic microcirculation and hepatic absorption spectra were examined in perfused rat liver.

MATERIALS AND METHODS

1. *Experimental animals.* Male Sprague–Dawley rats, weighing 245 to 290 g, were employed in this study. They were provided with water and standard laboratory chow *ad libitum*.

2. *Liver perfusion.* Fed rats were anesthetized with sodium pentobarbital (45 mg/kg i.p.) and the livers were isolated and perfused with Krebs–Henseleit buffer (pH 7.4, 37°) saturated with 95% O_2 + 5% CO_2 in a hemoglobin-free, nonrecirculating system [14]. For perfusions in the anterograde direction, perfusate was pumped into the liver via a cannula inserted into

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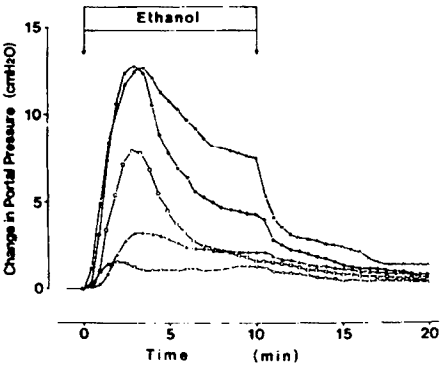

Fig. 1. Effect of ethanol on portal pressure in perfused rat liver. Livers from fed rats were perfused at a constant flow rate (36 mL/min) in anterograde direction with hemoglobin-free, Krebs–Henseleit buffer (pH 7.4, 37°) saturated with 95% O_2 + 5% CO_2. Portal pressure was measured by the manometer every 30 sec. Infusion of ethanol is indicated by arrows. Each symbol indicates mean value from four livers. Ethanol concentration: —○—, 25 mM; —▲—, 50 mM; —□—, 100 mM; —●—, 200 mM; —△—, 400 mM.

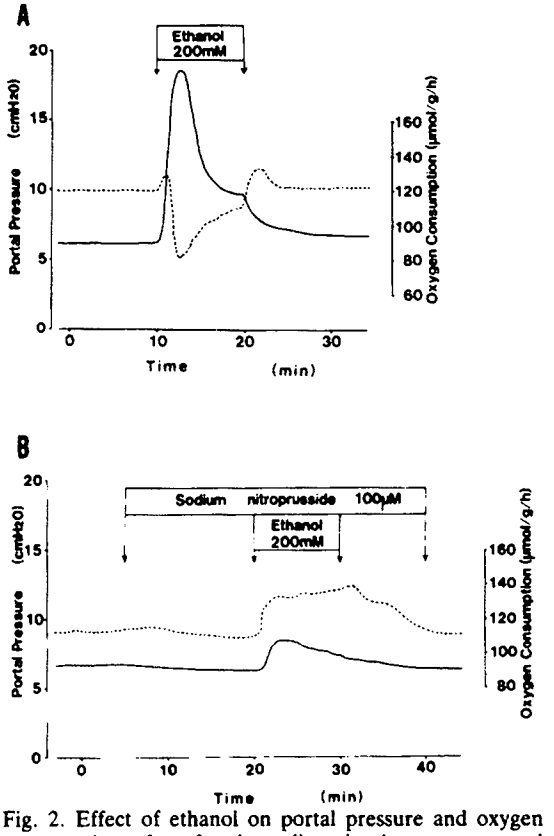

Fig. 2. Effect of ethanol on portal pressure and oxygen consumption of perfused rat liver in the presence and absence of sodium nitroprusside. Livers from fed rats were perfused at a constant flow rate (36 mL/min) in anterograde direction in the presence (B) and absence (A) of sodium nitroprusside. Simultaneous measurements of portal pressure (solid line) and oxygen consumption (dotted line) were performed employing the manometer and Clark-type oxygen electrode as described in Materials and Methods, respectively. Infusion of sodium nitroprusside (100 μM) and ethanol (200 mM) is indicated by arrows.

the portal vein at a constant flow rate (36 mL/min), and effluent perfusate was collected via a cannula placed in the suprahepatic inferior vena cava. For perfusions in the retrograde direction, the direction of flow was reversed from hepatic vein to portal vein. In both perfusions, the infrahepatic inferior vena cava and the hepatic artery were ligated.

3. *Measurements of oxygen consumption.* Oxygen concentrations in the influent and effluent perfusate were monitored continuously employing a Clark-type oxygen electrode. Oxygen consumption of the liver was calculated from the influent–effluent oxygen concentration difference, the flow rate and the wet weight of the liver.

4. *Measurements of portal pressure.* Portal pressure was measured with the level of buffer in an open vertical capillary (i.d. = 2 mm) at the inflow every 30 sec. Zero point was determined at the end of each experiment by the fluid level in the capillary when perfusate was pumped out through an influent cannula without liver at a constant flow rate.

5. *Spectrophotometric analysis.* Hepatic absorption spectrum was analysed in a reflection manner using organ reflectance spectrophotometry described previously [10]. In brief, the flexible optical fiber bundle (i.d. = 6 mm, Sumitomo Electronic Ind., Osaka, Japan) was placed in gentle contact with the surface of the perfused liver with a micromanipulator. A single fiber-optic bundle consisted of two fiber bundles for illuminating and reflecting light. The spectrophotometer used was equipped with a microcomputer and linear array silicon photodiode (512 KU, Matsushita Tsushin Co., Osaka, Japan) [15]. In this system, a reflectance spectrum was obtained in a short time (0.08 – 0.16 sec) and the spectra were stored in a memory system. The computer was programmed to subtract a reference spectrum of the liver.

(a) Estimation of redox state of mitochondrial respiratory cytochromes: difference absorption spectra of the surface liver were obtained using the spectrophotometer described above between the absence and presence of infused ethanol [16].

(b) Spectral analysis of hepatic tissue hemoglobin: rinsed erythrocytes suspension was infused into perfused liver (final hematocrit of perfusate: 1%), and the difference spectrum of regional hepatic tissue was obtained between the livers perfused with and without erythrocyte suspensions. The concentration of hemoglobin in hepatic tissue was estimated by the difference in absorption between 569 and 650 nm, $\Delta Er_{569-650}$, since there is a good correlation between the local tissue hemoglobin concentration and the difference in the spectral intensity between these two wavelengths [16]. The $\Delta Er_{569-650}$ is considered to reflect the regional hepatic vascular bed [10, 16].

The reagent, sodium nitroprusside, was purchased from the Sigma Chemical Co., (St Louis, MO). The results are expressed as mean ± SEM unless otherwise stated. Statistical analysis was performed using the Student's *t*-test. Differences with a P value of <0.05 were considered statistically significant.

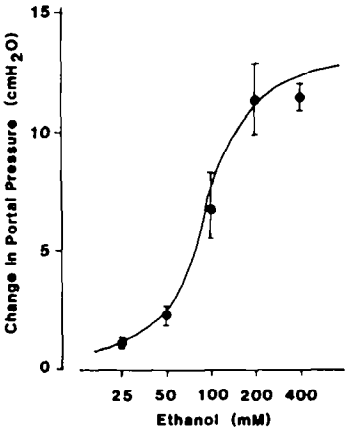

Fig. 3. Concentration–response curve for the effect of ethanol on change in portal pressure in perfused rat liver. Livers were perfused in anterograde direction in experiments depicted by Fig. 1. Data show the change of portal pressure at 3 min after starting of ethanol infusion when it reached maximal. Each symbol of closed circle and bar indicates mean ± SEM from four livers.

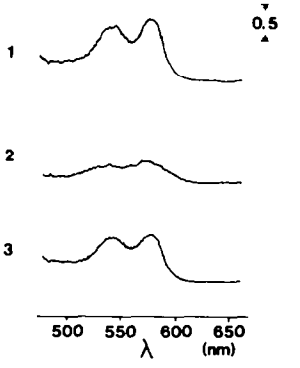

Fig. 4. Typical recording showing the effect of ethanol infusion on hepatic microcirculation of perfused rat liver as assessed spectroscopically. Livers from fed rats were perfused in anterograde direction. Absorption spectrum of the liver was analysed in a reflection manner using organ reflectance spectrophotometry from 475 to 660 nm. Rinsed erythrocyte suspension was infused into the influent (final hematocrit of perfusate, 1%). The difference spectrum of the liver in the presence and absence of erythrocytes in the perfusate was obtained. The erythrocytes were detected from the hemoglobin absorption in the difference spectrum. (1) before infusion of ethanol; (2) 3 min after starting of infusion of ethanol (200 mM); (3) 15 min after termination of ethanol infusion. λ, wavelength.

RESULTS

Effect of ethanol on portal pressure in perfused rat liver

Portal pressure was 6.4 ± 0.4 cm H_2O (N = 20) before infusion of ethanol. After starting of ethanol infusion the portal pressure increased immediately, and reached the maximal level at 3 min. Portal pressure gradually decreased thereafter, became a plateau and was kept at a higher level than that prior to ethanol infusion. It returned to the basal level when ethanol infusion was terminated (Figs 1 and 2). The maximal increase in portal pressure following infusion of ethanol was significantly higher than the basal value and was dependent on the concentration of ethanol infused. The value showed maximal (11.5 cm H_2O) at the concentration of ethanol more than 200 mM (half-maximal concentration, 90 mM) (Figs 1 and 3).

Sodium nitroprusside (100 μM), a direct acting vasodilator, produced little change in portal pressure before infusion of ethanol, but it diminished the increase in portal pressure following infusion of ethanol at 200 mM. The maximal change of portal pressure in the presence of sodium nitroprusside appeared at 3 min after infusion of ethanol and was significantly smaller than that in the absence of sodium nitroprusside (2.2 ± 0.8 cm H_2O, N = 4 vs 11.4 ± 1.5 cm H_2O, N = 4, $P < 0.01$) (Fig. 2).

Effect of ethanol on hepatic microcirculation of perfused rat liver.

Hepatic microcirculation of perfused rat liver was assessed by the absorption spectra of hemoglobin obtained randomly at the surface of the liver. Prior to infusion of ethanol, two absorption peaks of oxyhemoglobin were seen at 542 and 577 nm in a range of 475 to 660 nm, indicating that the erythrocytes were flowing well at the surface liver. However, in the presence of ethanol (200 mM), the hemoglobin signal diminished markedly in many portions of the surface liver perfused in anterograde direction and returned to the basal level when ethanol infusion was terminated (Fig. 4). Ethanol (200 mM) significantly decreased the index of the regional hepatic tissue hemoglobin concentration by 73% as an average at 3 min after starting of ethanol infusion. By contrast, ethanol (200 mM), when infused in retrograde manner, significantly increased the index of regional hepatic tissue hemoglobin concentration by 24% (Table 1).

Effect of ethanol infusion of oxidation–reduction of mitochondrial respiratory cytochromes in perfused rat liver

Effect of ethanol infusion on the absorption spectra of the superficial layer of the liver perfused in either anterograde or retrograde direction was analysed to examine whether redox change of mitochondrial respiratory cytochromes may occur after the load of ethanol. In anterograde perfusions, infusion of ethanol at more than 50 mM reduced the mitochondrial respiratory chain cytochromes $c + c_1$, $b(b_K + b_T)$ and aa_3, as evidenced by the appearance of the absorption peaks at around 550, 560 and 604 nm, respectively. When infusion of ethanol was terminated, the absorption peaks disappeared, and the difference spectrum returned to that of the control prior to the ethanol load. When the livers were subsequently perfused with the buffer saturated with 95% N_2 + 5% CO_2, the spectral peaks reappeared at around 550, 560 and 604 nm, which were essentially the same as those observed in the load of high concentration of ethanol.

Table 1. Effect of ethanol on the index of the regional hepatic tissue hemoglobin concentration (Δ $Er_{569-650}$) in rat liver perfused in either anterograde or retrograde direction

Direction	Δ $Er_{569-650}$ (O.D.) Before ethanol infusion	During 200 mM ethanol infusion
Anterograde	0.86 ± 0.05	0.23 ± 0.03*
Retrograde	0.72 ± 0.01	0.89 ± 0.04*

Livers from fed rats were perfused in either anterograde or retrograde direction. Hepatic spectra were obtained as described in the legend of Fig. 4. The index of regional hepatic tissue hemoglobin concentration before or 3 min after starting of ethanol infusion was obtained from the difference of spectral intensities between 569 and 650 nm (Δ $Er_{569-650}$). Values represent means ± SEM for four livers perfused in anterograde and five livers perfused in retrograde direction.

* $P < 0.01$ vs before ethanol infusion.

Table 2. Effect of ethanol infusion on the reduction of mitochondrial respiratory cytochrome $c + c_1$ and cytochrome aa_3 in perfused rat liver

Ethanol	Reduction of cyt $c + c_1$	Reduction of cyt aa_3
50 mM (N = 4)	0.023 ± 0.005 (29 ± 7%)	0.017 ± 0.005 (24 ± 8%)
100 mM (N = 4)	0.041 ± 0.008 (52 ± 9%)	0.031 ± 0.011 (41 ± 11%)
200 mM (N = 4)	0.058 ± 0.004 (67 ± 6%)	0.046 ± 0.004 (60 ± 6%)
400 mM (N = 4)	0.052 ± 0.005 (67 ± 9%)	0.036 ± 0.006 (51 ± 7%)
N_2 (N = 16)	0.083 ± 0.004 (100%)	0.075 ± 0.004 (100%)

Livers from fed rats were perfused in anterograde direction. Ethanol was infused into the influent at the concentration of 50, 100, 200 or 400 mM. Change of cytochrome $c + c_1$ or aa_3 to the reduced form at 3 min after starting of ethanol infusion was determined as described in Materials and Methods. The value represents the change of OD from the control value (ethanol, 0 mM) and is expressed as mean ± SEM. N_2, perfusion with the buffer saturated with 95% N_2 + 5% CO_2 in the absence of ethanol.

The redox level of hepatic cytochrome $c + c_1$ or aa_3 was estimated by the difference of spectral intensities between 550 and 540 nm ($\Delta Er_{550-540}$) or 604 and 620 nm ($\Delta Er_{604-620}$), respectively [16]. Infusion of ethanol at concentrations of 50–400 mM increased the reduction level of cytochromes $c + c_1$ and aa_3 in parallel and in a concentration-dependent fashion at 3 min after starting of ethanol infusion (Table 2). The reductions of these cytochromes showed a parabolic correlation with the increase in portal pressure following infusion of ethanol (Fig. 5). In the presence of sodium nitroprusside (100 μM), the reduction of cytochromes $c + c_1$ and aa_3 was significantly inhibited (0.010 ± 0.002 and 0.008 ± 0.001 vs 0.058 ± 0.004 and 0.46 ± 0.004, N = 4, $P < 0.01$, respectively). In contrast, infusion of ethanol in retrograde fashion did not induce any spectral changes at around 550 nm, 560 nm and 604 nm.

Effect of ethanol of oxygen consumption in perfused rat liver

In anterograde perfusions with ethanol at 200 mM, the hepatic oxygen consumption showed a transient increase followed by a striking decrease. The maximal decrease in oxygen consumption was observed at 3 min after starting ethanol infusion. The oxygen consumption returned to the basal level after termination of ethanol infusion (Fig. 2). The change in oxygen consumption at 3 min after infusion of ethanol was concentration-dependent. The maximal decrease of oxygen consumption was 45 μmol/g/hr (41%) as compared to that of steady state prior to ethanol load. Half-maximal concentration of ethanol was 90 mM (Fig. 6). As shown in Fig. 7, there was a reciprocal relationship between the changes in portal pressure and oxygen consumption following infusion of ethanol. The hepatic oxygen consumption was not decreased but increased by ethanol (200 mM) in the presence of sodium nitroprusside (Fig. 2).

DISCUSSION

This study aims to evaluate our hypothesis that the ethanol induces hepatic vasoconstriction, leading to hepatic microcirculatory disturbance, with resulting hepatic hypoxia. Portal vein pressure of the rat liver perfused at a constant flow rate was increased by infusion of ethanol in a concentration-dependent fashion (Figs 1 and 3). The increase in portal pressure was shown to be due to hepatic vasoconstriction caused by ethanol, since this increase in portal pressure was diminished in the presence of sodium nitroprusside, a direct acting vasodilator (Fig. 2). There was an "escape"

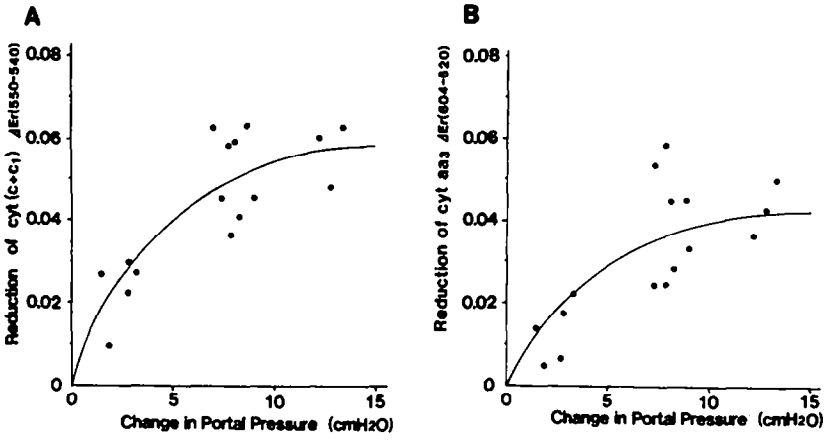

Fig. 5. Relationship between the changes in portal pressure and reduction of cytochromes $c + c_1$ and aa_3 following ethanol infusion in perfused rat liver. Livers from fed rats were perfused at a constant flow rate (36 mL/min) in anterograde direction. Ethanol was infused into the influent at the concentration of 50, 100, 200 or 400 mM. Portal pressure was measured by the manometer every 30 sec. Absorption spectrum of the liver after infusion of ethanol was obtained as described in Materials and Methods. Change of cytochrome $c + c_1$ (A) or cytochrome aa_3 (B) to reduced form in perfused liver following ethanol infusion was determined by the difference of spectral intensities between 550 nm and 540 nm (Δ $Er_{550-540}$) or 604 nm and 620 nm (Δ $Er_{604-620}$), respectively. Data were obtained at 3 min after ethanol infusion from 16 separate experiments.

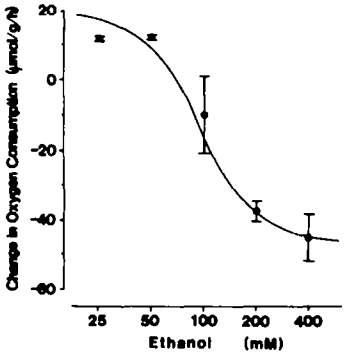

Fig. 6. Concentration–response curve for the effect of ethanol on change in oxygen consumption of perfused rat liver. Livers from fed rats were perfused in anterograde direction in experiments depicted by Fig. 1. Data were obtained at 3 min after ethanol infusion when the increase in portal pressure reached maximal. Each symbol of closed circle and bar indicates mean ± SEM from four livers.

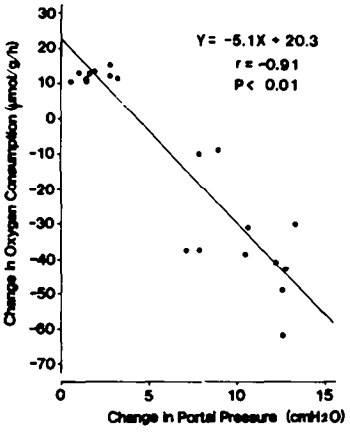

Fig. 7. Relationship between changes in portal pressure and oxygen consumption following ethanol infusion in perfused rat liver. Livers from fed rats were perfused in anterograde direction in experiments depicted by Fig. 1. Data were obtained at 3 min after ethanol infusion from 20 separate experiments.

phenomenon of the ethanol-induced increase in portal pressure (Fig. 1). Possible explanations for that are: first, factors regulating the vasodilation and constriction are limited in concentration and its continuity is also limited; second, the sensitivity of vasoconstriction to ethanol falls in the time course of ethanol infusion. Further investigations are required regarding this mechanism.

Effect of ethanol on the redox change of mitochondrial respiratory cytochromes in the liver was randomly examined on the surface of the liver. When ethanol was infused into the influent, the spectral peaks appeared at around 550, 560 and 604 nm, indicating a reduction of mitochondrial respiratory cytochromes $c + c_1$, b and aa_3. Ethanol changed mitochondrial respiratory cytochromes $c + c_1$ and aa_3 to the reduced form in a concentration-dependent fashion in a range from 50 to 400 mM (Table 2). The difference spectrum of the liver in the presence of 200 and 400 mM of ethanol was quite similar to that obtained under anoxic condition, indicating that the ethanol reduced the respiratory cytochromes $c + c_1$ and aa_3 quite similarly as that under the anoxic condition. Therefore, it seemed

that the ethanol at concentrations over 50 mM developed focal hypoxia in the liver perfused normally from portal vein, resulting in the complete reduction of the respiratory cytochromes in some hepatocytes, at least, in superficial layers of the liver. Since the reduction of the respiratory cytochromes was associated with the decrease in hepatic oxygen consumption (Table 2, Fig. 6), it is unlikely that the increase in hepatic oxygen consumption following ethanol infusion developed deficient of oxygen supply to some hepatocytes in the perfused liver. Therefore, ethanol seemed to disturb the oxygen delivery to some hepatocytes in the liver perfused in anterograde direction, with resulting focal hepatic hypoxia. There was a parabolic correlation between the reduction of the respiratory cytochromes following ethanol infusion and the increase in portal pressure (Fig. 5). Moreover, the reduction of the cytochromes was inhibited by sodium nitroprusside (100 μM). Thus, it was indicated that the ethanol-induced hepatic vasoconstriction developed hepatic hypoxia via disturbance of oxygen delivery to some hepatocytes. Indeed, the regional hepatic tissue hemoglobin concentration, estimated by the difference absorption spectra of the liver, decreased significantly following infusion of 200 mM of ethanol (Table 1, Fig. 4). The regional hepatic tissue hemoglobin concentration is a good index of hepatic tissue blood volume averaged within the area of probe tip (6 mm in diameter), and reflects vascular beds consisted chiefly of sinusoids and venules [10, 16]. Therefore, the decrease of absorption intensity of hemoglobin indicates that infusion of ethanol diminished the vascular bed at the superficial layer of the liver. It also indicates that the ethanol disturbed the microcirculation, at least in part, at surface liver tissue. When trypan blue was infused into the influent, it was homogenously visualized by its staining in the absence of ethanol. Whereas, trypan blue showed a heterogeneous staining on the liver surface in the presence of 200 mM ethanol and the staining changed to homogeneous pattern after termination of ethanol infusion (data not shown). It reveals that the ethanol disturbed hepatic microcirculation, and suggests that some portions of the liver had little or no flow, consequently disturbing the oxygen delivery to some hepatocytes. Thus, it is conceivable that the ethanol infused at a concentration of more than 50 mM caused the vasoconstriction and disturbed the circulation especially at the surface liver and that the mitochondrial respiratory chain cytochromes in some hepatocytes were reduced due to oxygen deficit.

The oxygen consumption was increased by the infusion of ethanol at concentrations of 25 or 50 mM compared to that in a steady state before ethanol infusion (Fig. 6). This finding is consistent with the previous observations [14, 17]. The inhibition of glycolysis after stimulated oxidation of ethanol has been demonstrated to account for the ethanol-induced increase in the oxygen consumption [14]. On the contrary, the ethanol at higher concentrations depressed the hepatic oxygen consumption in a concentration-dependent fashion (Fig. 6). The depression of the hepatic oxygen consumption was due to inhibition of mitochondrial respiration, since the depression was associated with the reduction of mitochondrial respiratory chain cytochromes aa_3 and $c + c_1$ (Table 2, Fig. 5). The change in oxygen consumption was associated with the increase in portal pressure (Fig. 3), and there was a significant reciprocal relationship between the change in portal pressure and oxygen consumption following infusion of ethanol (Fig. 7). In addition, sodium nitroprusside diminished not only the ethanol-induced increase in the portal pressure but the depression of oxygen consumption following infusion of ethanol (Fig. 2). These results imply that the ethanol-induced hepatic vasoconstriction caused the reduction of hepatic oxygen consumption via disturbance of oxygen delivery to hepatocytes and resulting hepatic hypoxia.

The exact vascular region where ethanol constricted was not clear in this study. In anterograde perfusions, the index of regional hepatic tissue hemoglobin concentration was markedly decreased by ethanol (200 mM). By contrast, it was significantly increased by ethanol (200 mM) in retrograde perfusions (Table 1). The result indicates that the ethanol increased hepatic vascular resistance mainly at presinusoidal regions, that is, the ethanol had a vasoconstrictive effect on hepatic vasculature presumably at presinusoidal regions. Recently, Yang *et al.* [13] reported that ethanol constricted an isolated canine hepatic portal vein *in vitro* in a dose-dependent fashion at the range of 43 to 430 mM. Jenkins *et al.* [12] reported that hepatic vascular resistance of rats increased in response to the increase in the rate of intravenous infusion of ethanol from 27 to 61 mM. Bravo *et al.* [11] also observed an increased hepatic vascular resistance in dogs following an intraportal injection of ethanol. These results are consistent with our finding that ethanol induced hepatic vasoconstriction in perfused rat liver.

Lieber and Sato [9] recently reported that intravenous infusion of ethanol at high level (55 mM) decreased hepatic oxygen utilization in the baboons, associated with a marked shift in the mitochondrial redox level in the liver and with the increase in splanchnic output of acetaldehyde. The ethanol infusion produced a change in neither splanchnic blood flow nor hepatic vein oxygenation in these baboons. Therefore, the impaired oxygen utilization was postulated mainly to be due to the increased acetaldehyde. In this case, on the other hand, the index of hepatic tissue hemoglobin concentration, assessed by reflectance spectroscopy on the liver surface, significantly decreased after the high ethanol dose. It indicates that the ethanol disturbed the microcirculation of the superficial liver tissue, which led to an impairment of hepatic oxygen utilization. The data also support our present finding that the high concentration of ethanol induced an impairment of hepatic oxygen consumption via hepatic microcirculatory disturbance and resulting focal hepatic hypoxia.

In this study, the ethanol induced hepatic vasoconstriction at concentrations from 25 to 400 mM in a concentration-dependent manner. Ethanol levels in peripheral blood of some human alcoholics have been reported to exceed 100 mM [18, 19]. The ethanol concentration in portal blood was approximately

double of that in the peripheral blood, when the acute administration of ethanol was performed by gastric tube in rats *in vivo* [20]. Thus, ethanol levels in portal blood of some alcoholics are assumed to be quite high after heavy drinking. Therefore, the ethanol concentrations by which hepatic vasoconstriction was induced, as shown in this study, could be seen in portal blood of alcoholics after heavy drinking. However, it is still obscure whether the ethanol can induce the necrosis of hepatocytes after such microcirculatory disturbance and focal hypoxia.

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