PHARMACOLOGY AND CELL METABOLISM

Ethanol Predominantly Constricts Pre-sinusoids of Isolated Perfused Livers of Rat, Guinea pig and Mouse

Toshishige Shibamoto1,*, Mikihiro Tsutsumi2, Chieko Ohmukai1, Yuhichi Kuda1, Wei Zhang1 and Yasutaka Kurata1

1Department of Physiology II, Kanazawa Medical University, Uchinada 920-0293, Japan and 2Department of Gastroenterology, Kanazawa Medical University, Uchinada 920-0293, Japan

*Corresponding author. Tel: +81-76-218-8104; Fax: +81-76-286-8010; E-mail: shibamo@kanazawa-med.ac.jp

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Abstract — Aims: Ethanol constricts hepatic vessels of isolated perfused livers of rats, but not dogs. However, it is not known whether ethanol constricts or dilates the hepatic vessels in other species such as guinea pigs and mice. In addition, the sites of hepatic vasoconstriction induced by ethanol were not known in rat livers. We therefore studied the effects of ethanol on the segmental hepatic vascular resistance and liver weight of mice, rats and guinea pigs. Methods: The isolated livers were porally perfused with diluted blood at constant flow. The sinusoidal pressure was measured by the double occlusion method and was used to determine the pre- and post-sinusoidal resistance. The change of liver weight was also measured. Ethanol was administered cumulatively into the perfusate to gain clinically relevant concentrations of 1–300 mM. Results: Ethanol dose dependently caused predominant pre-sinusoidal constriction in livers of all three species. When compared with the livers of the guinea pigs and rats, the mouse livers were the weakest in response. Dose-dependent decreases in liver weight and bile flow accompanied predominant pre-sinusoidal constriction in guinea pigs and rats. Conclusion: Ethanol predominantly constricts pre-sinusoids in rat, guinea pig and mouse livers, although the mouse liver response was much weaker. Ethanol-induced pre-sinusoidal constriction is accompanied by reduction of liver blood volume in guinea pigs and mice.

INTRODUCTION

The microcirculation of the hepatic sinusoids plays a crucial role in the integrity of liver function (Chun et al., 1994). Ethanol may influence liver function via its vasoactive effects on sinusoidal circulation. It is well known that perfusion with ethanol causes an increase in portal venous pressure (Ppv) in rat livers (Hijioka et al., 1991; Matsumoto et al., 2000; Oshita et al., 1993). In contrast, in isolated perfused canine livers, ethanol reduces Ppv (Faro et al., 1998). These findings suggest that there are species differences in the hepatic vascular response to ethanol. However, it is not known whether ethanol increases or decreases Ppv in guinea pigs and mice.

Furthermore, the hepatic vascular segments in which ethanol may exert a vasoconstrictor or vasodilator action remain unknown. We have clarified the changes in the segmental hepatic vascular resistance induced by various vasoactive substances in isolated perfused livers of rats (Shibamoto et al., 2005a), guinea pigs (Ruan et al., 2004a) and mice (Liu et al., 2007), by measuring the sinusoidal pressure with the hepatic vascular occlusion method (Yamaguchi et al., 1994). This method would be useful in determining the hepatic vascular segment as a target of ethanol actions.

The purpose of the present study was to determine the effects of clinically relevant concentrations of ethanol (1–300 mM) on the pre- and post-sinusoidal resistances and liver weight in isolated perfused mouse, rat and guinea pig livers, by measuring the sinusoidal pressure with the vascular occlusion method (Yamaguchi et al., 1994).

MATERIALS AND METHODS

Male Balb/c mice (n = 10; 27 ± 2 g), male Sprague-Dawley rats (n = 10; 251 ± 20 g) and male Hartley guinea pigs (n = 10; 317 ± 18 g) were used in this study. Animals were maintained at 23°C and under pathogen-free conditions on a 12/10 hours dark/light cycle, and received food and water ad libitum. The experiments conducted in the present study were approved by the Animal Research Committee of Kanazawa Medical University. We followed the principles of laboratory animal care (NIH publication No. 85-23, Revised 1996). All animals were purchased from Japan SLC (Hamamatsu, Japan).

Isolation and preparation of liver

The animals were anesthetized with intraperitoneal pentobarbital sodium and were mechanically ventilated with room air. The methods for isolation and preparation of liver for perfusion were previously described (Liu et al., 2007; Ruan et al., 2004a; Shibamoto et al., 2005a). After laparotomy, the hepatic artery was ligated; the bile duct was cannulated with a polyethylene tube in rats and guinea pigs. At 5 min after heparinization (500 U kg⁻¹) via the right carotid artery for rats and guinea pigs or via the intra-abdominal inferior vena cava (IVC) for mice, blood (8–9 ml in rats or guinea pigs or 0.8 ml in mice) was withdrawn through the carotid arterial or IVC catheter. The IVC above the renal veins was ligated, and the portal vein was cannulated with a stainless steel cannula. After thoracotomy, the suprahepatic caval IVC was cannulated through a right atrium incision with a stainless steel cannula; then portal perfusion was begun with the heparinized autologous blood diluted with 5% bovine albumin (Sigma-Aldrich, St. Louis, MO, USA) in Krebs solution (118 mM NaCl, 5.9 mM KCl, 1.2 mM MgSO₄, 2.5 mM CaCl₂, 1.2 mM NaH₂PO₄, 25.5 mM NaHCO₃ and 5.6 mM glucose) at hemocrit of 12% for rat and guinea pig livers and 6% for mouse livers. The livers were rapidly removed, suspended from the isometric transducer (TB-652T, Nihon-Kohden, Tokyo, Japan) and weighed continuously throughout the experiment.

The liver was perfused in a recirculating manner at a constant flow rate through the portal vein with blood that was...
Measurement of hepatic vascular pressure and resistance

The Ppv and hepatic venous (Phv) pressures were measured with pressure transducers (TP-400T, Nihon-Kohden) attached by a sidearm to the appropriate cannula with the reference points at the hepatic hilus. The portal blood flow rate (Qpv) was measured with an electromagnetic flow meter (MFV 1200, Nihon-Kohden), the flow probe of which was positioned in the inflow line in rat and guinea pig livers. In mouse livers, Qpv was measured manually by collecting outflow perfusate for 1 min just before the baseline measurement. The hepatic sinusoidal pressure was measured using double occlusion pressure (Pdo) method (Yamaguchi et al., 1994): both the inflow and outflow lines were simultaneously and instantaneously occluded for 13 s using the solenoid valves, after which Ppv and Phv rapidly equilibrated to an identical pressure, which was Pdo. The principle of the double occlusion method to estimate the sinusoidal pressure is derived from the concept of the mean circulating filling pressure of the systemic circulation (Rothe, 1993). Actually, Pdo values were obtained from the digitized data of Ppv and Phv using an original program (LIVER software, Biomedical Science, Kanazawa, Japan). The total portal–hepatic venous (Rt), pre-sinusoidal (Rpre) and post-sinusoidal (Rpost) resistances were calculated as follows:

\[
R_t = \frac{P_{pv} - P_{hv}}{Q_{pv}}
\]

\[
R_{pre} = \frac{P_{pv} - P_{do}}{Q_{pv}}
\]

\[
R_{post} = \frac{P_{do} - P_{hv}}{Q_{pv}}
\]

Data recording

The Ppv, Phv, Qpv, liver weight and bile weight were monitored continuously and displayed through a thermal physiograph (RMP-6008, Nihon-Kohden). All outputs were also digitized via the analog-digital converter at a sampling rate of 100 Hz. These digitized values were also displayed and recorded using a personal computer for later determination of Pdo.

Experimental protocol

Hepatic hemodynamic parameters were observed for at least 20 min after the start of perfusion until an isogravimetric state (no weight gain or loss) was obtained by adjusting Qpv and the height of the reservoir at a Phv of 0–1 cmH2O. After the baseline measurements, ethanol was administered as a bolus into the reservoir in a cumulative manner to gain the concentrations of 1–300 mM. In some livers with ethanol at 10 or 100 mM, Ppv did not return to the pre-injection levels; in this case, further high concentrations were not examined. Therefore, the high concentration of 100 or 300 mM was studied separately in several livers. In each liver, double occlusion maneuvers were performed at baseline and maximal venoconstriction (i.e. when Ppv reached the peak) after an injection of ethanol.

Statistics

The results are expressed as mean ± SD. Statistical analyses were performed with analysis of variance, and a P-value of <0.05 was considered significant. When a significant difference was obtained, post hoc analysis was performed with the Bonferroni post test method.

RESULTS

The basal hepatic hemodynamic variables

Table 1 shows basal hemodynamic variables of isolated perfused mouse, rat and guinea pig livers. Basal Ppv values were similar among three species, but Ppv values were different: Qpv were 22 ± 2, 34 ± 5 and 41 ± 4 ml min⁻¹ per 10 g liver weight for mouse, rat and guinea pig livers, respectively. Therefore, the order of basal Rt was mouse > rat > guinea pig. The Rpost-to-Rt ratios were also different; highest in the mouse (0.49 ± 0.03), intermediate in the guinea pig (0.40 ± 0.05) and lowest in the rat (0.32 ± 0.04).

The hepatic responses to ethanol

Figures 1–3 show representative responses to ethanol in a liver from a rat, guinea pig and mouse, respectively. Figure 4 shows the summarized data of changes in the hepatic vascular pressure and resistance, liver weight and bile flow rate at various concentrations of ethanol (1–300 mM). In livers of all three species, ethanol induced hepatic venoconstriction in a dose-dependent manner, as reflected by a significant

<table>
<thead>
<tr>
<th>Number of animals</th>
<th>Mouse</th>
<th>Rat</th>
<th>Guinea pig</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Wet liver weight (g)</td>
<td>1.4 ± 0.1***</td>
<td>8.3 ± 1.2**</td>
<td>10.6 ± 0.9*</td>
</tr>
<tr>
<td>Ppv (cmH2O)</td>
<td>8.0 ± 1.0</td>
<td>7.3 ± 0.6</td>
<td>7.5 ± 0.5</td>
</tr>
<tr>
<td>Phv (cmH2O)</td>
<td>0.2 ± 0.3</td>
<td>0.4 ± 0.2</td>
<td>0.5 ± 0.4</td>
</tr>
<tr>
<td>Pdo (cmH2O)</td>
<td>4.0 ± 0.4***</td>
<td>2.6 ± 0.3**</td>
<td>3.3 ± 0.2*</td>
</tr>
<tr>
<td>Qpv (ml min⁻¹·per 10 g liver)</td>
<td>22 ± 2***</td>
<td>34 ± 5</td>
<td>41 ± 4</td>
</tr>
<tr>
<td>Rt (cmH2O ml⁻¹·per min·per 10 g liver)</td>
<td>0.36 ± 0.04***</td>
<td>0.21 ± 0.03**</td>
<td>0.17 ± 0.04</td>
</tr>
<tr>
<td>Rpre (cmH2O ml⁻¹·min·per 10 g liver)</td>
<td>0.19 ± 0.02**</td>
<td>0.14 ± 0.03</td>
<td>0.10 ± 0.02</td>
</tr>
<tr>
<td>Rpost (cmH2O ml⁻¹·min·per 10 g liver)</td>
<td>0.17 ± 0.02**</td>
<td>0.07 ± 0.01</td>
<td>0.07 ± 0.02</td>
</tr>
<tr>
<td>Rpost-to-Rt ratio</td>
<td>0.49 ± 0.03***</td>
<td>0.32 ± 0.05</td>
<td>0.40 ± 0.04</td>
</tr>
</tbody>
</table>

Values are given as means ± SD.

Ppv, portal venous pressure; Phv, hepatic venous pressure; Pdo, double occlusion pressure; Qpv, portal blood flow rate; Rt, total vascular resistance; Rpre, pre-sinusoidal resistance; Rpost, post-sinusoidal resistance.

*P < 0.017 vs. rat.

**P < 0.017 vs. guinea pig.
increase in Ppv. In rat and guinea pig livers, Ppv reached the similar peak levels at 300 mM (22 ± 3 cmH2O for rats and 24 ± 4 cmH2O for guinea pigs). In contrast, the responsiveness to ethanol was the weakest in mouse livers: Ppv increased only to 11 ± 2 cmH2O at 300 mM. Phv did not change in any livers studied because the livers were perfused at constant flow. Ethanol caused a significant increase in Pdo in all of three species, as shown in Fig. 4. These findings indicate that ethanol-induced increase in the Ppv-to-Pdo gradient, an indicator of Rpre (Equation 2) was greater than that in the Pdo-to-Phv gradient, an indicator of Rpost (Equation 3). Indeed, as shown in Fig. 4, although ethanol dose dependently increased Rpre and Rpost, at 300 mM ethanol the increases of Rpre were higher than those of Rpost in all species. These results indicate that ethanol predominantly constricts the pre-sinusoids in mouse, rat and guinea pig livers. The order of peak values of Rpre and Rpost at 300 mM ethanol was guinea pigs > rats > mice (Fig. 4).

In response to ethanol, the liver weight decreased in a dose-dependent manner in rats and guinea pigs, with a maximum decrease at 300 mM being −0.3 ± 0.1 and −0.5 ± 0.1 g per 10 g liver weight for rat and guinea pig, respectively. In mouse, the liver weight did not alter significantly at any dose of ethanol studied.

The basal level of the bile flow rate of rat and guinea pig livers were 0.006 ± 0.0006 and 0.03 ± 0.01 g min⁻¹ per 10 g liver weight, respectively. The bile flow rate decreased in a dose-dependent manner in both rat and guinea pig livers after ethanol administration (Fig. 4). At 300 mM ethanol, the decrease in bile flow in rats (to 69 ± 7% of
DISCUSSION

We determined the effects of ethanol on vascular resistance in pre- and post-sinusoids and liver weight in isolated perfused livers of rat, guinea pig and mouse. The main finding is that ethanol predominantly constricted pre-sinusoids over post-sinusoids in all the three species studied in a dose-dependent manner. Furthermore, the hepatic vеноconstriction by ethanol was similar in magnitude in rat and guinea pig, but much weaker in mouse. Ethanol-induced pre-sinusoidal constriction was accompanied with reduction in liver weight in guinea pigs and rats.

Hepatic segmental vascular response to ethanol depends on animal species. Ethanol causes dilation of portal veins in perfused canine livers with a constant flow (Faro et al., 1998). In contrast, ethanol concentrations from 25 to 400 mM have been shown to cause hepatic vеноconstriction in rats (Hijioka et al., 1991; Oshita et al., 1993). We confirmed these findings on rats and further demonstrated that ethanol at 10–300 mM produced vеноconstriction, predominantly in pre-sinusoids in mice and guinea pigs. The concentrations of ethanol studied were clinically relevant since the ethanol concentrations up to 100 mM (90–460 mg/dl) are encountered during binge drinking, and blood ethanol concentrations as high as 300 mM have been also encountered (Berlid and Hasselbalch, 1981; Johnson et al., 1982; O’Neill et al., 1984).

Here we report for the first time that perfused livers of mice and guinea pigs showed vеноconstrictive responses to
ethanol. Similar to rat livers, guinea pig livers showed pre-
dominant pre-sinusoidal vasoconstriction at 300 mM ethanol.
However, at 10–100 mM, the increases in the pre- and post-
sinusoidal resistances were similar in guinea pig livers. This
indicates that ethanol constricts substantially post-sinusoids
as well at lower concentrations. Indeed, the post-sinusoids of
guinea pig liver may substantially contract in response to
vasoactive substances such as histamine (Liu et al., 2007; Ruan et
al., 2004b; Shibamoto et al., 2005b; Zhao et al., 2009), angiotensin II
(Zhao et al., 2009), endothelin-1 (Zhao et al., 2009), histamine
(Shibamoto et al., 2005b) and platelet-activating factor (Liu et
al., 2007) in both ex vivo isolated perfused livers and in vivo anesthetized preparations. In this
respect, we have recently reported that there is only a
small amount of vascular smooth muscle in mouse portal
venules, as demonstrated by the immunohistochemical
studies (Zhao et al., 2009). Thus, the weak response of
mouse hepatic vasoconstriction to ethanol could be
explained in part by poor distribution of smooth muscle cells
in mouse portal veins.

The mechanism of the ethanol-induced hepatic pre-
sinusoidal vasoconstriction is not known. Ethanol could con-
tract hepatic vessels directly or indirectly, i.e. via the other
vasoactive substances, which may act on hepatic vascular
smooth muscles. Oshita et al. (1993) using isolated perfused
rat livers with infusion of ethanol reported that endothelin-1
antiserum inhibited the increase in Pvp by 45–80% and
thereby concluded that endothelin-1 is responsible for
ethanol-induced rat hepatic vasoconstriction. Acetaldehyde,
a metabolite of ethanol could be a candidate. However, there
is no evidence that acetaldehyde causes hepatic vasoconstric-
tion. On the contrary, Kawahara et al. (1993) reported that
acetaldehyde inhibits contraction of hepatic stellate cells.
Prostaglandins synthesized from arachidonic acid by cyto-
chrome P4502E1 (CYP2E1) may cause hepatic vasoconstriction.
Although it is well known that ethanol induces
CYP2E1, it takes a minimum of 3 days to induce CYP2E1
in the rat liver treated with 5% ethanol through a liquid diet
(Tsutsumi et al., 1993). In this study, ethanol was adminis-
tered as a bolus into the liver, so the chance of induction of
CYP2E1 is very remote.

On the other hand, there is substantial evidence that
ethanol produces vasoconstriction by acting directly on the
smooth muscle itself in the aorta and the cerebral artery
(Altura and Altura, 1982; Gordon et al., 1995; Knych, 1987;
Werber et al., 1997). Although further mechanisms are not
fully known, ethanol-induced inhibition of the large-
conductance, Ca²⁺-activated K⁺ channels of smooth muscle
cells is proposed (Bukiya et al., 2009). However, these
mechanistic investigations have not been performed on
hepatic vasculature.

In rat and guinea pig livers, ethanol causes predominant
pre-sinusoidal constriction and liver weight reduction (as
shown in Fig. 4). The liver weight loss indicates a decrease
in liver blood volume. As for the mechanism of decrease in
the liver blood volume during pre-sinusoidal constriction, we
assume that constriction of pre-sinusoids may induce decrease
in the blood flow of the corresponding downstream sinus-
oids, and their blood volume (Takano et al., 2010).

In conclusion, ethanol at 10–300 mM concentrations pre-
dominantly constricted pre-sinusoids over post-sinusoids in
rats, guinea pigs and mice, although constriction of the
mouse liver was much weaker. Ethanol-induced pre-
sinusoidal constriction is accompanied with reduction of
liver blood volume in guinea pigs and rats.

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