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Alcohol Induces Relaxation of Rat Thoracic Aorta and Mesenteric Arterial Bed

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Abstract — Aims: The aim of this study was to investigate the effect of alcohol on rat artery and its underlying mechanism. Methods: The tension of isolated Sprague-Dawley rat thoracic aortic rings and the pressure of rat mesenteric arterial beds perfused with different concentrations of alcohol (0.1–7.0‰) were measured. Results: At resting tensions, alcohol caused a concentration-dependent relaxation on endothelium-denuded aortic rings precontracted with KCl (6 × 10⁻⁶ mol/L) or phenylephrine (PE, 10⁻⁶ mol/L), and this effect was most evident on rings at a resting tension of 3 g. Alcohol induced much less vasodilation on endothelium-intact rings. Alcohol inhibited the CaCl₂-induced contraction of endothelium-denuded aortic rings precontracted with KCl or PE. Incubation of rings with dantrolene (5 × 10⁻⁵ mol/L), a ryanodine receptor blocker, or 2-aminoethyl diphenylborinate (7.5 × 10⁻⁵ mol/L), an IP₃ receptor blocker, attenuated the vasodilating effect of alcohol on rings precontracted with PE. Alcohol also concentration-dependently relaxed rat mesenteric arterial beds precontracted with KCl (6 × 10⁻⁶ mol/L) or PE (10⁻⁶ mol/L), which was more potent on endothelium-denuded than on endothelium-intact beds. Conclusions: Alcohol has a vasodilating effect on rat artery depending on the resting tension. Both extracellular and intracellular Ca²⁺ mobilization of vascular smooth muscle cells are involved in the vascular effect of alcohol.

INTRODUCTION

The relationship between alcohol and the cardiovascular system is complex and not fully elucidated. Numerous epidemiological studies report that low-level ingestion of alcohol has beneficial consequences (Dyer et al., 1980; Friedman and Kimball, 1986; Klatsky et al., 1992; Djoussé et al., 2002; Mann and Folts, 2004), particularly against coronary heart disease and ischaemia-reperfusion injury. However, excessive alcohol intake has long been associated with cardiovascular disorders, including cardiomyopathy, coronary artery disease and stroke (Friedman and Kimball, 1986; Kasper et al., 1994; Thun et al., 1997; Truelsen et al., 1998; Mann and Folts, 2004).

As regards the effect of alcohol on blood pressure, studies are inconsistent (Khetarpal and Volier, 1981; Hashimoto et al., 2001; Tawakol et al., 2004; Puddey and Beilin, 2006). It is reported that drinkers of alcoholic beverages have higher blood pressure (Klatsky et al., 1977; Gillman et al., 1995; Puddey and Beilin, 2006) or are at a higher risk for developing hypertension (Khetarpal and Volier, 1981; Fuchs et al., 2001). On the other hand, most studies on the acute effect of alcohol on arteries show a vasodilatation (Agewall et al., 2000; Hashimoto et al., 2001; Tawakol et al., 2004). Besides, it is reported that chronic alcohol ingestion markedly attenuates α₁-adrenergic-induced contraction (Sahna et al., 2000). Thus, the true effect of alcohol on vascular tone is uncertain and the mechanism has not been elucidated.

Ca²⁺ plays an important role in the regulation of vascular tone. The initiation of contraction in vascular smooth muscle is due to a rise of free cytosolic Ca²⁺ concentration, which is caused by Ca²⁺ entry via ion channels in the plasma membrane and by Ca²⁺ release from the sarcoplasmic reticulum (SR) via ryanodine receptors and IP₃ receptors. Extracellular Ca²⁺ enters the intracellular space mainly through voltage-dependent Ca²⁺ channels (VDCs) and hormone-mediated receptor-operated Ca²⁺ channels (ROCAs) (Adams et al., 1989; Kanneganti and Halpern, 1996). We hypothesized that the vascular effect of alcohol may involve the mobilization of Ca²⁺.

Based on this background, we were prompted to investigate the vascular effect of alcohol on isolated rat artery and the relationship between this effect and the resting tension of the blood vessel, and to determine its underlying mechanism.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats weighing 230–260 g were obtained from the Laboratory Animal Center of the Chinese Academy of Medical Sciences, Hangzhou, China. All procedures were approved by the Ethics Committee for the Use of Experimental Animals in Zhejiang University, Hangzhou, China.

Chemicals and drugs

Phenylephrine (PE), acetylcholine (ACH), ethylene glycol-bis[β-aminoethyl ether]-N,N,N,N-tetraacetic acid (EGTA), dantrolene and 2-aminoethyl diphenylborinate (2-APB) were purchased from Sigma Chemical Co. (Saint Louis, MO, USA). Alcohol was obtained from the Hangzhou Long March Chemical Plant (Hangzhou, China). All other chemicals were of analytical grade and were purchased from Huadong Chemical (Hangzhou, China).

Preparation of thoracic aortic rings

Rats were anaesthetized with chloral hydrate (0.4 g/kg, intraperitoneal) and sacrificed by decapitation. The chest was opened and the thoracic aorta was rapidly removed and placed in a 4°C Krebs-Henseleit (K-H) solution [mmol/L: NaCl 118.4, KCl 4.7, CaCl₂ 1.25, KH₂PO₄ 1.2, MgSO₄ 1.2, NaHCO₃ 25.0 and glucose 11.0 (pH 7.4)]. After the removal of the superficial connective tissue, the aorta was cut into rings 3–4 mm in length and mounted in 5.0 mL organ baths containing the K-H...
solution. The bath solution was maintained at 37°C and bubbled continuously with a gas mixture of 95% O2 and 5% CO2. Aortic rings were equilibrated for 90 min at resting tensions ranging between 1 and 4 g. Isometric tension was measured with force isometric transducers connected to a data acquisition system (MedLab, Nanjing Medease Co., Ltd, Nanjing, China). In some rings, the endothelium was mechanically removed by gentle rubbing with moistened cotton. The removal of endothelial cells was confirmed by the loss of ACh-induced relaxation (Kamata and Makino, 1997; Wang et al., 2006; Qian et al., 2006).

Preparation of the mesenteric arterial bed

Rats were anaesthetized and then given an intravenous injection of HP (1000 units/kg). The abdominal cavity was opened by a mid-line incision through the linea alba, and the superior mesenteric artery was immediately cannulated and perfused with the physiological salt solution [PSS, mmol/L: NaCl 119.0, KCl 4.7, CaCl2 1.6, MgSO4 1.2, NaH2PO4 1.2, NaHCO3 25.0 and glucose 11.1 (pH 7.4)]. The small intestine was then separated from the arterial bed along the intestinal wall. The mesenteric arterial bed was transferred to a jacketed chamber that was kept at 37°C and perfused with warm (37°C), oxygenated (95% O2 and 5% CO2) PSS at a constant flow rate of 5 mL/min through the cannula, using a peristaltic pump, and was equilibrated for 60 min. Perfusion pressure was recorded with a pressure transducer and displayed on a data acquisition system (PowerLab, ADInstruments Pty Ltd, Sydney, Australia). The perfusion pressure was expressed in mmHg (Kamata and Makino, 1997). In some experiments, the arterial bed was perfused with Triton X-100 (1%) for 30 s to remove the endothelium (Kamata et al., 1996). The removal of endothelial cells was confirmed by the loss of ACh-induced relaxation.

Functional studies of thoracic aortic rings

Both endothelium-denuded and endothelium-intact aortic rings at different resting tensions were pre-contracted with KCl (6×10^{-2} mol/L) or PE (10^{-6} mol/L) and a cumulative concentration–response curve to alcohol at concentrations ranging between 0.1‰ and 7.0‰ was performed after the KCl- or PE-induced contraction reached a plateau. The vascular response was expressed as a percentage of the 'plateau' constriction evoked by KCl or PE.

The effect of alcohol (3.0‰) was also measured for 20 min on endothelium-denuded aortic rings previously incubated for 30 min with dantrolene (a ryanodine receptor blocker, 5×10^{-5} mol/L) or 2-APB (an IP3 receptor blocker, 7.5×10^{-5} mol/L).

In another set of experiments, after incubation with alcohol (3.0‰) for 30 min in a Ca^{2+}-free medium (containing 5×10^{-2} mol/L EGTA), endothelium-denuded aortic rings were pre-contracted with KCl (6×10^{-2} mol/L) or PE (10^{-6} mol/L) and the contractile response to CaCl2 at concentrations ranging between 0.25×10^{-3} and 5.00×10^{-3} mol/L was evaluated.
Contraction was expressed as a percentage of the maximal contraction in response to KCl or PE (Jiang et al., 2005; Qian et al., 2006).

**Functional studies of the mesenteric arterial bed**

The perfusion pressure of both the endothelium-denuded and endothelium-intact mesenteric arterial bed was increased by either KCl (6 × 10^{-2} mol/L) or PE (10^{-5} mol/L). After the pressure reached a plateau, a cumulative concentration–response curve to alcohol (0.1–7.0 %) was performed. The vascular response was expressed as a percentage of the ‘plateau’ constriction evoked by KCl or PE.

All drugs were added to a scaled reservoir that was kept at 37°C and bubbled continuously with a gas mixture of 95% O_2 and 5% CO_2, and from which PSS was perfused to the mesenteric arterial bed.

**Statistical analysis**

Data are expressed as mean ± standard deviation. Statistical differences were assayed by one-way ANOVA followed by the Newman–Keuls test or two-way ANOVA followed by the Bonferroni post-test. Differences were considered significant at \( P < 0.05 \).

**RESULTS**

**Alcohol induced relaxation of thoracic aortic rings**

Alcohol had no effect on the baseline tension of aortic rings. On endothelium-denuded aortic rings at different resting tensions (1.0–4.0 g), pre-contracted with KCl (6 × 10^{-2} mol/L) or PE (10^{-5} mol/L), alcohol (0.1–7.0 %) caused a concentration-dependent relaxation, with a maximum relaxation reached at 3.0 % (Fig. 1 A). This effect was most evident when the aortic rings were at a resting tension of 3 g (Fig. 2). Alcohol induced more relaxation in rings pre-contracted with KCl than those pre-contracted with PE (\( p_D^2 \) 3.286 ± 0.580 versus 3.048 ± 0.362, \( E_{\text{max}} \) 22.63 ± 4.22 % versus 15.02 ± 6.60 %, \( n = 8 \)).

The effect of alcohol on endothelium-intact aortic rings was much weaker than on endothelium-denuded ones (\( E_{\text{max}} \) 10.90 ± 6.56 % versus 22.63 ± 4.22 %, \( P < 0.01 \), \( n = 6–8 \), on rings pre-contracted with KCl; \( E_{\text{max}} \) 8.25 ± 3.30 % versus 15.02 ± 6.60 %, \( P < 0.05 \), \( n = 6–8 \), on rings pre-contracted with PE) (Fig. 1 B, C and D).

**Alcohol induced relaxation of the mesenteric arterial bed**

Alcohol (0.1–7.0 %) had no effect on the baseline perfusion pressure of the mesenterial arterial bed, but caused a concentration-dependent relaxation in endothelium-denuded arterial bed pre-contracted with KCl (6 × 10^{-2} mol/L) or PE (10^{-5} mol/L) (Fig. 3 A). Alcohol induced less relaxation in the endothelium-intact arterial bed (\( E_{\text{max}} \) 10.81 ± 1.69 % versus 19.32 ± 1.97 %, \( P < 0.01 \), \( n = 4 \), in the arterial bed pre-contracted with KCl; \( E_{\text{max}} \) 6.14 ± 7.88 % versus 18.56 ± 6.04 %, \( P < 0.05 \), \( n = 4–5 \), in the arterial bed pre-contracted with PE) (Fig. 3 B, C and D).

**Effect of alcohol on CaCl_2-induced relaxation**

In a Ca^{2+}-free medium, CaCl_2 (0.25×10^{-3} to 5.00 × 10^{-3} mol/L) induced a concentration-dependent contractile response in endothelium-denuded aortic rings pre-contracted with KCl (6×10^{-2} mol/L) or PE (10^{-6} mol/L), at a resting tension of 3 g. Pre-incubation of aortic rings with alcohol (3.0 %) for 30 min inhibited the contractile response to CaCl_2 and shifted the concentration–response curve downward. This effect was more potent on rings pre-contracted with KCl than on those pre-contracted with PE (\( E_{\text{max}} \) 33.32 ± 8.35 % versus 19.01 ± 7.73 %; \( P < 0.01 \), \( n = 8 \)) (Fig. 4).

**Effects of pre-incubation with dantrolene or 2-APB**

Alcohol (3.0 %) induced relaxation in endothelium-denuded aortic rings pre-contracted with PE (10^{-6} mol/L), at a resting tension of 3 g. Pre-incubation of rings with dantrolene (5 × 10^{-5} mol/L) and 2-APB (7.5 × 10^{-5} mol/L) decreased the vasodilating effect of alcohol (Fig. 5).

**DISCUSSION**

The results of this study showed that alcohol caused concentration-dependent relaxation in both isolated rat thoracic
aorta and mesenteric arterial bed, consistent with several studies reporting that alcohol depresses PE-induced constriction (Strickland and Woolees, 1988), relaxes arteries (Agewall et al., 2000; Hashimoto et al., 2001; Tawakol et al., 2004), reduces vascular resistance and increases cerebral blood flow (Stendel et al., 2006). On the other hand, unchanged blood pressure or increased tension in response to alcohol has also been described (Chan and Sutter, 1983; Utkan et al., 2007). Reasons for these differences in vascular responses to alcohol might be explained by different experimental conditions, including the type of arteries, the methods used for vascular studies, different concentration of alcohol used and the duration of alcohol treatment.

Alcohol induced more relaxation in endothelium-denuded blood vessels than in endothelium-intact ones, suggesting that the vasodilating effect of alcohol is independent of endothelium and it acts directly on the vascular smooth muscle cells to reduce tone. Vascular smooth muscle uses Ca$^{2+}$ as the trigger for contraction. Ca$^{2+}$ influx via channels in the plasma membrane and Ca$^{2+}$ release from SR are the major sources (Adams et al., 1989; Kanneganti and Halpern, 1996; Wray et al., 2005).

Findings obtained from previous studies seem to indicate that VDC blockers, such as nimodipine or verapamil, attenuate the vascular effect of alcohol in cerebral blood vessels (Altura et al., 1983; Zhang et al., 1993), suggesting that the effect of alcohol may be mediated by its influence on Ca$^{2+}$ availability. Contraction induced by high K$^{+}$ is due to membrane depolarization, leading to Ca$^{2+}$ influx via VDCs (Godfraind et al., 1986), while the contractile effect of PE is mediated by a first component response, increased Ca$^{2+}$ influx through ROCs, and a second component response, Ca$^{2+}$ release from the SR (Ehrlich and Watras, 1988). In the present study, alcohol induced relaxation in arteries pre-contracted with KCl, and clearly reduced the Ca$^{2+}$-induced contraction in aortic rings exposed to KCl. These results indicate that alcohol probably acts by inhibiting the VDCs. Moreover, alcohol also significantly relaxed the artery and decreased the Ca$^{2+}$-induced contraction in aortic rings pre-contracted with PE, suggesting that alcohol reduces the efficiency of alpha-adrenoceptor activation and attenuates Ca$^{2+}$ influx through ROCs as well. In the present study, alcohol exhibited a greater relaxant effect against contraction induced by high KCl than by PE, indicating that VDCs on the plasma membrane are much more influenced by alcohol than ROCs.

There are two main kinds of Ca$^{2+}$ channels in SR membrane, ryanodine receptors and IP$_3$ receptors. PE, upon binding to its receptor, stimulates the formation of IP$_3$, which binds to and opens the IP$_3$ receptor channels, induces the release of internal Ca$^{2+}$ from SR, and causes a contraction of the artery (Ehrlich and Watras, 1988). It was reported that IP$_3$-induced intracellular Ca$^{2+}$ release after stimulation with PE does not contribute to the response of alcohol-treated aortas (Horowitz et al., 1996; Tirapelli et al., 2006). However, in our study, when aortic rings were pre-incubated with 2-APB, a
membrane-permeable reagent to block IP₃ receptors (Soulsby and Wojcikiewicz, 2002; Snetkov et al., 2003; Lamont and Wier, 2004; Ng et al., 2007) and store-operated Ca²⁺ influx (Prakriya and Lewis, 2001; Zhou et al., 2007), the vasodilating effect of alcohol was reduced. A similar phenomenon occurred when we pre-incubated the tissue with dantrolene, an agent that inhibits the release of intracellular Ca²⁺ via ryanodine receptors (Du et al., 2005; Ng et al., 2007). These results indicate that alcohol-induced relaxation involves inhibition of Ca²⁺ release from intracellular stores via IP₃ receptors and ryanodine receptors, and possible inhibition of store-operated Ca²⁺ influx.

It was a very interesting phenomenon that the relaxation induced by alcohol was most evident when the resting tension of aortic rings was 3 g, rather than 1–2 g, which is usually applied in functional studies of rat thoracic aortic rings (Maeso et al., 1996; Kamata and Makino, 1997; Arun et al., 2005; Maeso et al., 1996). Thus, appropriately increasing the resting tension of blood vessels may enhance the vasodilating effect of alcohol. Stretch-activated (SA) channels have been found in a wide variety of cells, including vascular smooth muscle cells and they are activated by stretch of the plasma membrane (Davis et al., 1992; Park et al., 2003, 2006). We hypothesize that since the resting tension of the aortic rings modulates SA channels, impacting the vascular tone and metabolism (Jackson, 2000; Iwasaki et al., 2003; Youn et al., 2006), alcohol may interact with SA channels in the vascular effect at different initial resting tension levels. This needs further investigation.

In addition, the doses of alcohol used in this study are consistent with those reported from other labs (Yang et al., 2001; Liu et al., 2004). Alcohol in this dose range acts on several types of ion channels, for example, the voltage-dependent Ca²⁺, Na⁺ and K⁺ channels, Ca²⁺-activated K⁺ channels and ATP-dependent K⁺ channels (Brodie and Sampson, 1990; Habuchi et al., 1995; Wu and Chao, 1995; Pagel et al., 2000; Yang et al., 2001; Kuhlmann et al., 2004; Sun et al., 2008). However, the non-specific effect of alcohol at high concentrations should be considered in investigating its vascular influence and the underlying ionic mechanisms.

In summary, the present work demonstrates that alcohol causes endothelium-independent vasorelaxation in both rat thoracic aorta and mesenteric arterial bed. This effect is dependent on the resting tension of blood vessels. Alcohol-induced relaxation is probably due to the inhibition of Ca²⁺ influx from extracellular space and Ca²⁺ release from the SR of vascular smooth muscle cells.

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