Direct effect of ethanol on human vascular function

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Tawakol, Ahmed, Torbjorn Omland, and Mark A. Creager. Direct effect of ethanol on human vascular function. Am J Physiol Heart Circ Physiol 286: H2468–H2473, 2004. First published February 5, 2004; 10.1152/ajpheart.01207.2003.—Epidemiological studies indicate that moderate ethanol consumption reduces cardiovascular mortality. Cellular and animal data suggest that ethanol confers beneficial effects on the vascular endothelium and increases the bioavailability of nitric oxide. The purpose of this study was to assess the effect of ethanol on endothelium-dependent, nitric oxide-mediated vasodilation in healthy human subjects. Forearm blood flow (FBF) was determined by venous occlusion plethysmography in healthy human subjects during intra-arterial infusions of either methacholine (0.3, 1.0, 3.0, and 10.0 mcg/min, n = 9), nitroprusside (0.3, 1.0, 3.0, and 10.0 mcg/min, n = 9), or verapamil (10, 30, 100, and 300 mcg/min, n = 8) before and during the concomitant intra-arterial infusions of ethanol (10% ethanol in 5% dextrose). Additionally, a time control experiment was conducted, during which the methacholine dose-response curve was measured twice during vehicle infusions (n = 5). During ethanol infusion, mean forearm and systemic alcohol levels were 227 ± 30 and 6 ± 0 mg/dl, respectively. Ethanol infusion alone reduced FBF (2.5 ± 0.1 to 1.9 ± 0.1 ml·dl^-1·min^-1, P < 0.05). Despite initial vasoconstriction, ethanol augmented the FBF dose-response curves to methacholine, nitroprusside, and verapamil (P < 0.01 by ANOVA for each). To determine whether this augmented FBF response was related to shear-stress-induced release of nitric oxide, FBF was measured during the coinfusion of ethanol and N^O-nitro-L-arginine (L-NNAME; n = 8) at rest and during verapamil-induced vasodilation. The addition of L-NNAME did not block the ability of ethanol to augment verapamil-induced vasodilation. Ethanol has complex direct vascular effects, which include basal vasoconstriction as well as potentiation of both endothelium-dependent and -independent vasodilation. None of these effects appear to be mediated by an increase in nitric oxide bioavailability, thus disputing findings from preclinical models.

In humans, ingestion of an alcoholic beverage acutely increases heart rate, cardiac output, and cardiac stroke volume (22, 23) and decreases blood pressure (23, 36). Furthermore, systemic administration of alcohol is associated with increases in forearm blood flow (FBF) and coronary blood flow (4, 22, 32). However, studies examining the effect of ethanol on NO-mediated vasodilation in humans have yielded inconsistent results (9, 17). Moreover, such prior observations of the hemodynamic effects of ethanol were made in the setting of oral or intravenous ethanol administration and hence are confounded by interactions of ethanol with the central nervous system (CNS).

The purpose of this study was to test the hypothesis that the vascular actions of ethanol in humans are mediated by endothelium-derived NO. FBF was measured in healthy human subjects during the intra-arterial infusion of endothelium-dependent and -independent vasodilators before and during concomitant intra-arterial administration of ethanol. Therefore, the local vasmotor effects of ethanol were assessed during inhibition of NO synthase.

METHODS

Patient population. Healthy subjects were recruited from the greater Boston area. Exclusion criteria included the following: smoking, systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg, serum cholesterol level above the 75th percentile for age and sex, history of vascular disease, diabetes, family history of premature coronary artery disease, or any clinical manifestation of atherosclerosis, such as coronary artery disease, peripheral artery disease, or carotid artery disease. The study protocol was approved by the Partners Joint Human Research Committee, and informed consent was obtained from each subject.

Experimental protocol. Each subject was studied in a 23°C temperature-controlled room in the fasting state. Nonsteroidal anti-inflammatory drugs, alcohol, and caffeine were prohibited for 12 h before study initiation. Under local anesthesia and sterile conditions, a 20-gauge polyethylene catheter was placed in the brachial artery of each subject for determination of blood pressure and for infusion of drugs. The vascular research laboratory was kept quiet, and the lights were dimmed. All subjects rested at least 30 min after catheter placement to establish a stable baseline before data collection. Venous occlusion plethysmography (described below) was employed to measure the FBF response to endothelium-dependent and -independent vasodilators during coadministration of ethanol or vehicle. Methacholine chloride (Provocholine, Roche Labs; Nutley, NJ), a congener of acetylcholine that acts by stimulating NO release from the vascular endothelium, was administered intra-arterially at doses of 0.3, 1.0, 3.0, and 10.0 mcg/min (n = 9). To distinguish abnormalities in endothelial function from those of vascular smooth muscle, sodium nitroprusside...
(Elkins-Sinn; Cherry Hill, NJ), which acts as a NO donor, was administered intra-arterially at doses of 0.3, 1.0, 3.0, and 10.0 mcg/min \((n = 9)\). To evaluate the direct smooth muscle effects of ethanol independent of NO, the calcium channel antagonist verapamil (American Reagent Laboratory; Shirley, NY) was administered intra-arterially at doses of 10, 30, 100, and 300 mcg/min \((n = 9)\). On each visit, only a single vasodilator was employed to test the mechanism by which ethanol affects FBF. Each vasoactive drug was infused for 5 min at a rate of 0.4 ml/min. A 5% dextrose solution served as the vehicle control for determination of baseline flows.

After the blood flow response to incremental doses of methacholine, nitroprusside, or verapamil was recorded, at least 45–60 min were allowed to pass to ensure reestablishment of basal conditions. Ethanol solution (10% ethanol in 5% dextrose) or vehicle was then infused in the ipsilateral brachial artery. The infusion rate for the ethanol solution was determined using the subject’s basal FBF, the concentration of ethanol in the solution, and the desired final blood concentration (200 mg/dl) as computational variables. The total intra-arterial infusion rate (ethanol solution plus dextrose solution) was maintained constant throughout the experimental series for each visit. In all cases, the total amount of ethanol infused during any one visit was \(<10\) gm ethanol, 1.e., less than the amount of ethanol in a single glass of wine). FBF measurements were repeated after ethanol had been infused intra-arterially for 30 min. The dose-response curve to the endothelium-dependent or –independent vasodilator drug was once again assessed, this time during coinfusion of ethanol. Additionally, a time control experiment was conducted (during which the methacholine dose-response curve was measured twice during vehicle infusions, \(n = 5\)).

**Contribution of NO to verapamil-stimulated vasodilation.** Numerous endothelium-independent vasodilators have been shown to elicit NO release (perhaps as a result of enhanced shear stress) \((2, 7, 19, 31, 44)\). As such, to determine whether NO contributes to verapamil-induced vasodilation in humans, FBF was measured during intra-arterial administration of verapamil and during the concomitant administration of vehicle vs. the NO synthase inhibitor \(N^\bullet\)-nitro-L-arginine (L-NAME; 2 mg/min over 10 min, Claflina; Läufeltingen, Switzerland, \(n = 5\)).

**Contribution of NO to ethanol-augmented vasodilation.** Thereafter, we sought to determine whether the vascular responses to ethanol were mediated by the release of NO. We employed verapamil as the vasodilator with which to test this hypothesis for three reasons. First, NO release can be attributed to NO, perhaps due to a direct effect of the drug can be measured. By measuring blood flow in the noninfused arm, confirmation can be made that the drug is not causing systemic effects. FBF was derived from the rate of change in forearm volume during venous occlusion and was recorded on a Gould physiological recorder. Measurements were expressed as milliliters per 100 ml of tissue per minute. Brachial arterial pressure was measured via an indwelling arterial cannula. The cannula was attached to a Gould pressure transducer, and the pressure measurements were recorded on a Gould physiological recorder. Forearm vascular resistance (FVR) was calculated as the ratio of mean blood pressure to FBF. To measure heart rate, the electrocardiogram was recorded and continually monitored during the experiments.

**Laboratory analyses.** Immediately after each ethanol infusion, blood samples were obtained from the ipsi- and contralateral antebrachial veins for plasma alcohol measurements. Plasma alcohol was measured by gas chromatography (Hewlett Packard; Palo Alto, CA). Systemic alcohol concentration was also measured at the bedside by means of a portable breath alcohol analyzer (Lifeloc Technologies; Denver, CO).

**Statistical analysis.** All data are presented as means ± SE. Group comparisons with respect to clinical characteristics were made with unpaired and two-tailed \(t\)-tests. FBF dose-response curves for each drug before and during the coadministration of ethanol were analyzed with two-way repeated-measures ANOVA. Followed by post hoc two-tailed \(t\)-tests adjusted with a Bonferroni correction for multiple comparisons. Statistical significance was accepted at the 95% confidence level \((P < 0.05)\).

**RESULTS**

**Patient population.** Twenty healthy subjects, including 12 men and 8 women (age, 29.5 ± 1.6 yr), participated in the protocols. Most subjects were studied in more than one protocol. The subjects’ mean total, LDL, and HDL cholesterol were 161 ± 6, 89 ± 4, and 52 ± 3 mg/dl, respectively.

**Blood alcohol concentrations.** Mean plasma alcohol concentration after ethanol infusion was 227 ± 30 mg/dl in the veins of the infused forearm. Mean systemic plasma alcohol concentration in the contralateral antebrachial veins was only 6 ± 0 mg/dl. The systemic ethanol concentration remained undetectable by breath analysis in all subjects.

**Effect of ethanol alone on hemodynamic parameters.** Intra-arterial ethanol infusion did not affect either heart rate \([\text{from 49 ± 5 to 53 ± 5 beats/min, } P = \text{not significant (NS)}]\) or mean arterial pressure \([\text{from 76 ± 2 to 77 ± 2 mmHg, } P = \text{NS}])\). However, ethanol reduced ipsilateral FBF \((\text{from 2.5 ± 0.1 to } 1.9 ± 0.1 \text{ ml} \cdot \text{dl}^{-1} \cdot \text{min}^{-1}, P < 0.01; \text{Fig. 1})\) and increased FVR \((\text{from 36 ± 3 to 47 ± 4 mmHg} \cdot \text{ml}^{-1} \cdot \text{dl}^{-1} \cdot \text{min} < 0.05)\). In contrast, ethanol infusion did not affect contralateral FBF.
(from 1.6 ± 0.1 to 1.7 ± 0.2 ml·dl\(^{-1}\)·min\(^{-1}\), P = NS) or FVR (from 49 ± 4 to 52 ± 6 mmHg·ml\(^{-1}\)·dl·min, P = NS).

**Effect of ethanol on endothelium-dependent and -independent vasodilation.** Ethanol infusion was associated with a significant upward shift in the ipsilateral FBF dose-response curves for each of the endothelium-dependent and -independent vasodilators (P < 0.05 by ANOVA for methacholine, nitroprusside, and verapamil; Fig. 2, A–C). During the highest dose of methacholine, FBF was 24.4 ± 1.9 ml·dl\(^{-1}\)·min\(^{-1}\) when vehicle was coinfused and 28.6 ± 1.4 ml·dl\(^{-1}\)·min\(^{-1}\) when ethanol was coinfused (P < 0.01). Similarly, during the highest dose of nitroprusside, FBF was 18.5 ± 1.4 ml·dl\(^{-1}\)·min\(^{-1}\) when vehicle was coinfused and 21.4 ± 2.3 ml·dl\(^{-1}\)·min\(^{-1}\) when ethanol was coinfused (P < 0.05). Likewise, during the highest dose of verapamil, FBF was 20.0 ± 2.6 ml·dl\(^{-1}\)·min\(^{-1}\) when vehicle was coinfused and 25.0 ± 2.6 ml·dl\(^{-1}\)·min\(^{-1}\) when ethanol was coinfused (P < 0.05). This effect was consistent; ethanol augmented FBF at the highest dose of vasodilator in 22 of 27 subjects. In congruence with these findings, FVR dose-response curves were reduced by a greater extent during ethanol infusion for each of the endothelium-dependent and -independent vasodilators (P < 0.05 by ANOVA for methacholine, nitroprusside, and verapamil). In contrast to those findings, in the time control experiment, (during which the FBF response to methacholine was measured twice during vehicle infusions), there was a nonsignificant reduction in FBF during the second series of measurements (P = NS; Fig. 2D). Furthermore, there were no significant changes in contralateral FBF or FVR during any of the intraarterial infusions.

**Effect of NO synthase inhibition.** The nitric oxide synthase inhibitor L-NAME did not affect resting heart rate (58 ± 8 vs. 58 ± 10 beats/min, P = NS) or mean arterial pressure (76 ± 11 vs. 75 ± 10 mmHg, P = NS). However, L-NAME was associated with an approximate 19% reduction in basal ipsilateral FBF (2.6 ± 0.4 to 2.1 ± 0.4 ml·dl\(^{-1}\)·min\(^{-1}\), P < 0.01; Fig. 3A). This effect was consistent; L-NAME reduced FBF at rest in eight of eight subjects. This was paralleled by a significant increase in FVR (30.4 ± 6.9 vs. 38.2 ± 10.1 mmHg·ml\(^{-1}\)·dl·min, P < 0.05). Furthermore, L-NAME infusion significantly attenuated the FBF dose-response curves to verapamil (P < 0.05 by ANOVA; Fig. 3A). The effect of L-NAME on verapamil-stimulated vasodilation was consistent; FBF at the highest dose of verapamil was reduced in eight of eight subjects.

During the coinfusion of L-NAME, ethanol did not further reduce resting FBF (2.3 ± 0.2 to 2.1 ± 0.3 ml·dl\(^{-1}\)·min\(^{-1}\), P = NS). Moreover, L-NAME did not blunt the ability of ethanol to upwardly shift the FBF dose-response relationship to verapamil (P < 0.05 by ANOVA; Fig. 3B). Despite the coinfusion of L-NAME, FBF increased in seven of eight subjects during ethanol and high-dose verapamil infusion.

**DISCUSSION**

The important new findings of this study are that 1) intraarterial ethanol infusion causes limb vasoconstriction at rest, and 2) ethanol enhances the FBF response to endothelium-dependent and -independent vasodilators via a mechanism that does not involve NO.

**Effect of ethanol on NO bioavailability.** This study tested the hypothesis that ethanol directly affects vasomotor function by acutely enhancing NO bioavailability. Such a finding would have highlighted a potential mechanism by which ethanol reduces cardiovascular mortality, because an increase in NO might lead to a reduction in the manifestations of atherosclerosis (via a decrease in vasoconstriction, leukocyte recruitment, platelet aggregation, and vascular smooth muscle proliferation). Indeed, animal and cellular studies have suggested that ethanol may increase NO bioavailability. Vascular strips taken from alcohol-fed rats relax more readily in response to acetylcholine (a stimulant for NO release) than vascular strips taken from control animals (18), and endothelial cells incubated with alcohol express more NO synthase mRNA (46) and greater l-citrulline conversion (a marker for NO production) (6).

Prior human studies that assessed the effect of ethanol on NO bioavailability have yielded conflicting results. The ingestion of red wine has been associated in an increase in serum NO (measured by fluorescent indicator) and improved flow-mediated vasodilation in some studies, whereas other studies found no improvement in NO-mediated vasodilation after red wine ingestion (9, 30). However, in each of those studies, measurements were made in response to systemic administration of ethanol. Ethanol is known to increase sympathetic

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**Fig. 2. A:** effect of EtOH on endothelium-dependent vasodilation. The effect of EtOH on FBF was determined in response to the endothelium-dependent vasodilator methacholine. Ethanol infusion was associated with a significant upward shift in the FBF dose-response curve for methacholine (P < 0.05 by ANOVA). **B:** effect of EtOH on endothelium-independent vasodilation. The effect of EtOH on FBF was determined in response to the endothelium-independent nitric oxide (NO) donor nitroprusside. Ethanol infusion was associated with a significant upward shift in the FBF dose-response curve for nitroprusside (P < 0.05 by ANOVA). **C:** effect of EtOH on NO-independent vasodilation. The effect of EtOH on FBF was determined in response to the endothelium-independent vasodilator verapamil. EtOH infusion was associated with a significant upward shift in the FBF dose-response curve for verapamil (P < 0.05 by ANOVA). **D:** time and vehicle control. To assess the effect of time on FBF, the dose-response curve to methacholine was determined twice in response to vehicle. There was a nonsignificant reduction in FBF during the second series of measurements (P = not significant by ANOVA).
activation via effects on the CNS (21, 22, 33, 45), thereby confounding the observations.

Thus, in the present study, we sought to examine the direct effect of ethanol on human vasomotor function. To accomplish this, ethanol was infused locally, via arterial catheter, into the vascular bed being studied, dosed to achieve a forearm concentration of 227 mg/dl, which is equivalent to that achieved after drinking four to six glasses of wine over 1 h. It is notable that the mean local blood alcohol concentration attained in this study is not frequently encountered during casual ethanol consumption (for comparison, the typical legal blood alcohol limit for driving an automobile in the United States is 100 mg/dl). Nonetheless, in the present study, systemic alcohol concentrations remained negligible. Therefore, the data reflect the direct effects of ethanol and are not confounded by the effects of ethanol on the CNS.

Effect of ethanol on resting blood flow. We found that ethanol acutely and directly reduces resting FBF and increases FVR in humans. This observation may be relevant to the noted association between chronic, heavy ethanol intake and hypertension (24, 25–27, 29). Prior observations suggested that alcohol induces pressor effects by sympathetic activation that appears to be centrally mediated (33). The vasoconstrictor effect observed in the present study is unlikely to result from centrally mediated changes in sympathetic tone, because systemic alcohol concentrations remained negligible during the course of this study and because contralateral FBF remained unchanged. The mechanism of the direct, acute vasoconstrictor effect of ethanol remains to be elucidated.

Ethanol augments vasodilation. Despite this initial vasoconstriction, ethanol increased the dilator response to methacholine, nitroprusside, and verapamil. This potentiating effect of ethanol was most apparent at the higher doses of vasodilators infused. In contrast, repeat infusion of vehicle (as a time control) did not change the vasodilator responses (a trend toward reduced vasodilation was instead seen).

The observation that ethanol augments both "endothelium-dependent" and "endothelium-independent" vasodilation does not, on its own, exclude the possibility that the mechanism of the effect of ethanol involves an endothelial release of NO. Indeed, several vasodilator drugs that are traditionally classified as endothelium independent have been shown to elicit NO release (2, 7, 19, 31, 44). In such cases, the initial endothelium-independent stimulus causes an increase in flow, which can potentially lead to additional increases in flow as a result of shear-stress-mediated NO release.

NO contributes to the dilator effect of verapamil. To test the hypothesis that NO release contributes to the vasodilator response of the classically endothelium-independent vasodilator verapamil, FBF was measured during intra-arterial administration of verapamil and during the concomitant administration of vehicle vs. l-NAME. During this series of experiments, we made the novel observation that NO synthase inhibition attenuates verapamil-induced vasodilation (Fig. 3A). This observation supports the hypothesis that NO release plays a role in the vasodilatory effect of verapamil in vivo. It follows, then, that although verapamil initially causes relaxation on vascular smooth muscle by inhibiting calcium channels, additional vasodilation may occur in vivo as a result of NO release (possibly resulting from shear-stress-mediated NO release).

Augmentation of vasodilation by ethanol occurs independently of NO. However, the same concentration of l-NAME did not prevent the ability of ethanol to augment vasodilation (Fig. 3B). Taken together, these data suggest that ethanol directly augments both endothelium-dependent and -independent vasodilation via a mechanism that is independent of NO. Moreover, the observation that NO does not play a role in the local vasoactive effects of ethanol does not directly conflict with the prior observations showing that alcoholic beverages enhance NO bioavailability. Indeed, prior studies suggest that it is the nonalcoholic components in wine that are responsible for the NO release (12, 40).

Potential mechanisms. Although these data demonstrate that NO does not play a significant role in the acute, direct effect of ethanol on vascular tone, the precise mechanisms by which ethanol altered vascular function in humans remains unexplained. It is plausible that the vascular effects of ethanol may result from its unique biophysical properties: the compact bipolar structure of ethanol allows it to readily intercalate within the lipid bilayer (3). As such, it may alter the actions of cell membrane channels (3), an ability that is thought to be responsible for some of the anesthetic properties of ethanol (3, 38, 39, 42). Therefore, an effect on membrane ion channels is an attractive potential mechanism that may explain the vasoactive effects of ethanol.

Study limitations. Caution should be exercised when extrapolating the direct, acute effects of pure alcohol to the effects of chronic, moderate consumption of alcoholic beverages. First, the vascular effect of pure ethanol may differ from the effect of alcoholic beverages, because some alcoholic beverages contain compounds that may have beneficial vascular actions that are independent of ethanol (12). Second, because by design systemic ethanol levels in this study were negligible, the findings of this study cannot account for potentially important indirect effects of ethanol or of by-products of ethanol metabolism. Third, the study examined only the acute effects of ethanol and may not reflect the chronic vasomotor effects of ethanol. Fourth, the lack of a significant fall in FBF after ethanol is added to l-NAME should be interpreted with caution, because the study was neither designed nor powered to examine the role
of NO in ethanol-mediated vasoconstriction at rest. Finally, the present study examined the acute, direct effects of ethanol on healthy subjects. As such, some of the observed findings (especially the magnitude of FBF change) may differ in a population with established atherosclerosis.

Clinical implications. The divergent direct vascular effects of ethanol reported in this study (resting vasoconstriction and potentiation of vasodilation) may, at first glance, seem counterintuitive. However, they coincide with two well-described clinical effects of alcohol consumption: hypertension (24, 25–27, 29) and potentiation of syncope (11). Indeed, a biphasic response to ethanol ingestion has been previously described (36).

In conclusion, the results of this investigation indicate that in healthy humans, ethanol acutely J) induces vasoconstriction at rest, and 2) augments endothelium-dependent and -independent vasodilation via a mechanism that does not involve NO. Further studies are needed to determine the mechanisms underlying the vascular actions of this commonly used compound.

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