Alcohol Suppresses Endothelium-Dependent Relaxation in Rat Mesenteric Vascular Beds

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The effects of prolonged infusions of ethanol on endothelium-dependent vasorelaxation induced by acetylcholine and adenosine triphosphate (ATP) and on endothelium-independent relaxation induced by papaverine were studied and compared in isolated perfused rat mesenteric artery preparations. Infusion of ethanol over 60 minutes at concentrations of 1.6, 4.7, 6.3, and 7.9 mg/ml caused concentration-related inhibition of norepinephrine-induced vasoconstriction. In preparations infused with 6.3 and 7.9 mg/ml, this effect reached a maximum after 10–20 minutes but had vanished by the end of the infusion; 1 hour after the end of the infusion, the effects of norepinephrine were potentiated by 71% and 108%, respectively. Acetylcholine-induced vasorelaxation (EC\textsubscript{50} 3.0 ng/ml in controls) was significantly reduced after 6.3 mg/ml ethanol infusion and totally abolished after 7.9 mg/ml ethanol infusion. ATP-induced vasorelaxation (EC\textsubscript{50} 180 ng/ml in controls) was also abolished after 7.9 mg/ml of ethanol infusion. By contrast, the vasorelaxant effects of papaverine were not affected by 7.9 mg/ml ethanol infusion. Light-microscopic examination revealed that the endothelial cells were present in ethanol-treated and in control mesenteric arterial beds. These observations indicate that ethanol suppresses endothelium-dependent vasorelaxation without apparent removal of the endothelial cells. The compromised relaxant capacity of the endothelium after ethanol and the resultant intensification of the vasoconstrictor response to norepinephrine may contribute to the development of vascular diseases such as hypertension and stroke. (Hypertension 1989;13:964–967)

Heavy alcohol consumption is known to be a risk factor for hypertension, stroke, and angina pectoris.\textsuperscript{1–3} The exact mechanisms involved in these ethanol-induced cardiovascular diseases are not yet fully understood. Recently the endothelium has been recognized as an important regulator of vascular tone.\textsuperscript{4,5} Vasorelaxation induced by acetylcholine and other vasoactive agents in preconstricted vessels is dependent on the presence of an intact endothelium (for review see Reference 6). Endothelial cells produce so-called endothelium-derived relaxing factor (EDRF), which acts on vascular smooth muscle by increasing cyclic guanosine monophosphate (cGMP).\textsuperscript{7,8} Removal of the endothelium results in an augmented vasoconstrictor response to various agents, including norepinephrine (NE).\textsuperscript{9,10} Similar potentiation of the constrictor effect of epinephrine has been reported after chronic ingestion of ethanol in rats.\textsuperscript{11} Recently, we found that the action of NE was likewise potentiated in isolated perfused mesenteric arterial beds (MABS) of rats infused with ethanol (unpublished observations). These similarities prompted us to examine the effects of ethanol on both endothelium-dependent vasorelaxation induced by acetylcholine and adenosine triphosphate (ATP) and endothelium-independent relaxation induced by papaverine.

Materials and Methods

Male rats of a Sprague-Dawley–derived strain of Rattus norvegicus (Tif:RAIf) were obtained from Tierfarm AG, Sisseln, Switzerland. Isolated superior mesenteric arteries from rats weighing 280–390 g were prepared according to a modification of the method of McGregor.\textsuperscript{12} The rats were anesthetized with ether, decapitated, and exsanguinated. The superior mesenteric artery was cannulated via the abdominal aorta, and the MAB was dissected free at its border with the intestine. MABs were perfused at a constant rate of 5 ml/min using a peristaltic pump (model 13/25, Istamec SA, Zürich, Switzerland) with a physiological perfusion solution (PPS) containing (mM): NaCl 136, KCl 2.5, NaHCO\textsubscript{3} 11.9, CaCl\textsubscript{2} 1.36, MgCl\textsubscript{2} 0.5, and Na\textsubscript{2}HPO\textsubscript{4} 0.42. The PPS was maintained at room temperature (20–

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22° C) and aerated with 95% O2 and 5% CO2, giving a pH of 7.2–7.4. Perfusion pressure, which under conditions of constant flow is proportional to vascular resistance, was recorded via a side arm of the arterial cannula, using Gould P23ID transducers (Gould Inc., Oxnard, California) and a Hellige recorder (Rüegge Medical, Baden, Switzerland).

Vasoconstriction was induced in MABs by bolus injection of 2 μg NE hydrochloride at intervals of 5 minutes throughout the experiment. After a stabilization period of 3 hours, ethanol solutions, made up in PPS, were infused at a rate of 0.1 ml/min for 60 minutes (final concentrations: 1.6, 4.7, 6.3, and 7.9 mg/ml). Only one concentration per artery was tested. Control preparations were infused with 0.1 ml/min PPS. A 60-minute recovery period was allowed before infusion of ascending concentrations of acetylcholine, ATP, or papaverine (10 min/concentration).

The relaxant effects of these agents are expressed as percent inhibition of the peak NE-induced vasoconstriction as compared with the initial control peak. At the end of the experiment two MABs per group were perfused with a fixative solution (glutaraldehyde 2.5% and sodium cacodylate 0.1 M) and postfixed in a solution of phosphate-buffered formalin. Approximately 20 2-μm-thick hydroxyethylmethacrylate sections of the arteries were prepared (internal diameters 180–250 μm), stained with toluidine blue, and examined under the microscope.

Statistical Methods

Results are expressed as mean±SEM. The data were analyzed statistically using analysis of variance followed by Bonferroni’s method. Values of p<0.05 were taken as statistically significant.

Results

In control MABs, the responses to NE tended to increase slightly during the experiment (Figure 1; n=9). MABs infused over 60 minutes with 1.6 mg/ml ethanol followed a similar trend (not shown). On the other hand, during the infusion of 4.7 mg/ml ethanol, the responses to NE decreased (8% after 30 minutes and 23% after 60 minutes; Figure 1) and continued to decline for up to 1 hour after the infusion was stopped (36%, n=7). Both 6.3 and 7.9 mg/ml ethanol infusion caused an initial inhibition of the NE response (41% and 63%, respectively, after 10–20 minutes, n=6 each), which lessened with time and had vanished by the end of the infusions (Figure 1). One hour after infusions of these two concentrations, the effects of NE were significantly potentiated in both sets of preparations (71% and 108%, respectively).

In control MABs, acetylcholine induced concentration-dependent inhibition of the response to NE (i.e., relaxation; IC50 3.0 ng/ml; Figure 2). The relaxant effect of acetylcholine in the preparations infused with 1.6 mg/ml ethanol was comparable with that seen in the control MABs (not shown). After 4.7 mg/ml ethanol infusion, the effect was slightly attenuated and after 6.3 mg/ml significantly suppressed (Figure 2). Pretreatment with 7.9 mg/ml ethanol totally abolished the response to acetylcholine (Figure 2).

ATP induced concentration-dependent relaxation in control MABs (IC50 180 ng/ml; n=7; Figure 3A). This effect was completely abolished in MABs perfused with 7.9 mg/ml ethanol (n=8; Figure 3A). By contrast, there was no difference in the relaxant effect of papaverine between control MABs and MABs treated with 7.9 mg/ml ethanol (IC50 0.42 vs. 0.39 μg/ml; n=4 each) (Figure 3B).

Microscopic examination revealed that the endothelial cells were present in all the ethanol-treated and control MABs.

Discussion

In these experiments, prolonged infusions of ethanol in the isolated perfused mesenteric artery of the rat suppressed the endothelium-dependent vasorelaxation induced by acetylcholine and ATP but not the endothelium-independent relaxation induced by papaverine.
FIGURE 2. Line graph of vasorelaxant effects of acetylcholine (ACh) in isolated perfused mesenteric beds of rat in control preparations, n=9 (●—●), and after a 60-minute infusion of ethanol 4.7 mg/ml, n=7 (○—○), 6.3 mg/ml, n=6 (□—□), or 7.9 mg/ml, n=6 (○—○). Relaxant effect is expressed as percent inhibition of norepinephrine-induced vasoconstriction (2-μg bolus at 5-minute intervals). Each concentration of ACh was infused for 10 minutes. Values are mean±SEM. In 6.3 mg/ml and 7.9 mg/ml ethanol-treated series, values obtained with ACh concentrations of 10^{-9} and above were significantly different from control values (p<0.05).

In addition, light-microscopic examination revealed that the endothelial cells were still present in all ethanol-treated mesenteric arteries, and no apparent difference from control MABs was demonstrable. By contrast, perfusion of mesenteric arteries with hypotonic solution for 5–10 minutes resulted in loss of the endothelium, suppression of acetylcholine-induced relaxation^{10,13} and, similar to the findings with ethanol described here, an increase of the vasoconstrictor response to NE.

Several other authors have reported that removal of the endothelium augments the responses to various pressor stimuli.^{4,9,10,13} Moreover, endothelium-dependent relaxation has been shown to be impaired in several animal models of hypertension^{14–16} and in atherosclerosis.^{4} How this impairment contributes to the development of hypertension or vascular diseases is not yet clearly understood. Studies in spontaneously hypertensive rats of two different ages indicate that impaired endothelium-dependent relaxation seen at an age when hypertension develops is the consequence and not the cause of the elevated blood pressure.^{4,17} Our present results, however, point to a possible involvement of endothelial dysfunction in alcohol-induced vascular diseases such as hypertension and stroke.

Numerous studies have documented the increased prevalence of hypertension in heavy drinkers or alcohol-dependent populations.^{1,3} In addition, heavy alcohol consumption has been recognized as an independent risk factor for stroke in men.^{3} Moreover, the same authors reported that the relative risk of stroke was lower in light drinkers than in abstainers, indicating that low concentrations of alcohol may exert protective effects, via vasodilation. Our findings demonstrate that alcohol has, in fact, a dual effect on the vascular system. Attenuation of the vasoconstrictor responses to NE was observed throughout the infusion of low concentrations but only during the first 30 minutes or so at high concentrations, after which the vasoconstrictor action of NE was gradually potentiated. This latter effect might be due to the suppression of endothelium-dependent relaxation by ethanol.

The mechanism involved in the suppression of endothelium-dependent relaxation by ethanol, however, needs further investigation. An effect of ethanol at the receptor level can very likely be excluded, as ethanol has no demonstrable influence on in vitro acetylcholine-receptor binding, even at concentrations higher than those used in this study.^{18} Ethanol has been shown to fluidize the bulk lipid of mem-

FIGURE 3. Panel A, line graph of vasorelaxant effects of adenosine triphosphate (ATP) in mesenteric vascular beds of rat in control preparations (●, n=7) and after 60-minute infusion of ethanol 7.9 mg/ml (○, n=8). Panel B, line graph of vasorelaxant effects of papaverine in mesenteric vascular beds of rat in control preparations (●, n=4) and after 60-minute infusion of ethanol 7.9 mg/ml (○, n=4). For details, see Panel A. Relaxant effect is expressed as percentage of norepinephrine-induced vasoconstriction (2-μg bolus at 5-minute intervals). Each concentration was infused for 10 minutes. Values are mean±SEM. All values in Panel A were significantly different from control values (p<0.05).
branes, with consequent alteration of cell function.19 Such a membrane effect at the level of the endothelial cells might prevent the formation and release of EDRF, leading similarly to removal of the endothelium, to potentiation of NE, and to suppression of endothelium-dependent relaxation. Our study does not, however, rule out the possibility that the membrane effect of ethanol may also promote the release of agents capable of preventing endothelium-dependent relaxation. Preliminary observations in our laboratory indicate that, in fact, endothelin, a vasoconstrictor peptide recently isolated from the culture supernatant of porcine endothelial cells,20 potentiates NE vasoconstriction and reduces, but does not abolish, endothelium-dependent relaxation.

This potentiation of NE vasoconstriction in response to prolonged infusion of ethanol could be responsible for the high incidence of stroke in heavy drinkers. A similar potentiation of the vasoconstrictor action of NE in mesenteric arterioles of rats after chronic ethanol ingestion has been reported.11 In addition, intra-arterial infusion of ethanol in a range of concentrations comparable with that used in our study reduced hand and forearm blood flow in humans.21 This effect could be the result of a direct vasoconstriction or of a loss of endothelium-dependent vasorelaxation, as shown in this study.

The implications of our findings in regard to alcohol-induced vascular diseases in humans need further investigation; it must also be borne in mind that, although the concentrations used in our study were roughly twice as high as those found in chronic heavy drinkers, the exposure time was only 1 hour. Studies on isolated vessels of heavy drinkers with a history of hypertension or stroke might afford a deeper insight into the role of the endothelium in these diseases.

In conclusion, these results indicate that ethanol can suppress endothelium-dependent vasorelaxation without apparently removing the endothelium. This compromised relaxant capacity of the endothelium after ethanol infusion and the consequently intensified vasoconstrictor response to NE may contribute to the development of alcohol-induced diseases such as hypertension and stroke.

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References


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