Ethanol Enhances Basal and Flow-Stimulated Nitric Oxide Synthase Activity In Vitro by Activating an Inhibitory Guanine Nucleotide Binding Protein

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ABSTRACT

The aim of this study was to determine the effect of ethanol on endothelial nitric oxide synthase (eNOS), the enzyme responsible for the production of the important vasoactive agent nitric oxide. The effect of ethanol (0.8–160 mM) on both basal and flow-stimulated eNOS activity was determined using cultured bovine aortic endothelial cells (EC). In “static” EC ethanol dose-dependently increased basal eNOS activity with a maximum response (~2.0-fold increase) achieved at 40 mM in the absence of any effect on cell viability or nitric oxide synthase protein expression. Pertussis toxin (PTX) pretreatment significantly inhibited the ethanol-induced increase in basal eNOS activity. EC exposed to steady laminar flow exhibited a flow- and time-dependent increase in eNOS activity. Ethanol significantly enhanced the laminar flow-induced eNOS response from 0.62 ± 0.1 to 1.06 ± 0.06 pmol [14C]citrulline/mg/min, a response that was inhibited by PTX. PTX-catalyzed ribosylation of Giα substrates, an index of G-protein functional activity, was increased in laminar flow-exposed EC compared with static controls and was further enhanced by ethanol treatment. Likewise, EC exposed to low (~0.5 dynes/cm²) and high (~12 dynes/cm²) pulsatile flow demonstrated increased eNOS activity, an effect that was associated with increased PTX-catalyzed ribosylation of Giα substrates. Ethanol enhanced the low flow response in a PTX-sensitive manner. These data demonstrate a stimulatory effect of ethanol on basal and flow-stimulated eNOS activity, mediated in part by a mechanism involving a PTX-sensitive G protein.

Over the past two decades important influences of ethanol on the cardiovascular system have been recognized. Several studies have demonstrated a consistent dose-response relationship between increasing alcohol consumption and decreasing incidence of coronary heart disease, despite an increase in mortality due to a large number of other diseases (Friedman and Kimball, 1986; Klatsky et al., 1992; Doll et al., 1994). Although the exact mechanisms of the peripheral vascular effects of ethanol are not yet fully understood, multiple interactions of ethanol with intracellular signal transduction processes have been demonstrated in a wide variety of cell types (Rabin and Molinoff, 1983; Hoffman and Tabakoff, 1990).

The endothelium is now recognized as an important regulator of vascular tone (Purchgott, 1983). Endothelial cells (EC) covering the inner surface of blood vessels are continuously exposed to hemodynamically imposed mechanical stress resulting from the flow of blood. EC respond to increases in flow by releasing vasodilator mediators, most notably endothelium derived relaxing factor, identified as nitric oxide (NO; Ranjan et al., 1995). NO is synthesized by the heme-containing enzyme nitric oxide synthase (NOS) from L-arginine in a reaction that produces stoichiometric amounts of L-citrulline (Moncada et al., 1991). Three isoforms of NOS have been identified by gene cloning. Two are constitutively expressed and one, the inducible NOS (iNOS) is expressed de novo in response to inflammatory cytokines (Moncada et al., 1991). Activation of NOS and release of NO results in stimulation of a soluble guanylyl cyclase leading to a profound increase in intracellular cGMP levels within most target cells (Moncada et al., 1991). NO plays a pivotal role in regulating blood flow by inhibiting smooth muscle contraction as well as platelet aggregation and adhesion (Moncada et al., 1991). In addition, NO has been shown to inhibit vascular smooth muscle cell proliferation, which plays an important role in the normal development of blood vessels, the pathogenesis of atherosclerosis, and the arterial response to injury (Schwartz and Liaw, 1993; Schwartz et al., 1995).

Previous studies, the majority in the central nervous system looking at iNOS, have provided data to support a specific
interaction between ethanol and the NOS/NO axis. Chen and LaBella (1997) demonstrated that alcohol noncompetitively inhibited rat brain NOS activity. Ethanol treatment blocked lipopolysaccharide-mediated induction of iNOS gene expression in the lung (Kolls et al., 1995) and in C6 glioma cells (Militante et al., 1997). However, in cultured vascular smooth muscle cells, ethanol potentiated interleukin-1β-stimulated iNOS expression (Durante et al., 1995). Although fewer investigators have examined the direct effect of ethanol on constitutive EC NOS, an ethanol enhancement of the NOS response to agonists such as bradykinin has previously been demonstrated in bovine pulmonary artery EC (Davda et al., 1993).

Heterotrimeric guanine nucleotide-binding proteins (G proteins) function as transducers of signals across the cell membrane by coupling diverse receptors to effectors and thus play a central role in signal transduction and cell biology (Levitzki and Bar-Sinai, 1991; Helmereich and Hofmann, 1996). Recent evidence suggests that inhibitory G proteins (Gi1,2,3), which are pertussis toxin (PTX)-sensitive, and Goq may play a role in both agonist- and shear stress-induced activation of endothelial nitric oxide synthase (eNOS) in EC (Cooke et al., 1991; Ohno et al., 1993). Interestingly, in the liver and central nervous system ethanol-induced alterations in the abundance and function of G proteins coupled to effectors such as adenylyl cyclase and phospholipase C have been demonstrated (Hoffman and Tabakoff, 1990; Iles and Nagy, 1995).

The aim of the present study was to determine the effect of ethanol on eNOS activity in cultured EC in vitro under both basal conditions and conditions of hemodynamic flow, the ubiquitous endothelial stimulus. To address this question we used bovine aortic EC in static cultures and those exposed to either laminar steady flow or pulsatile flow. We report here that ethanol enhances both basal and flow-stimulated eNOS activity by a mechanism involving a PTX-sensitive G protein.

**Experimental Procedures**

**Materials.** Dowex (AG 50 WX8, 200–400 mesh) was purchased from Sigma Chemicals (St. Louis, MO). [14C]Nicotinamide adenine dinucleotide (800 Ci/mmol) was obtained from New England Nuclear (Boston, MA). Antibodies specific for eNOS, iNOS, Gi1,2,3, Gi3, and Goq were purchased from Transduction Laboratories (Lexington, KY). Anti-rabbit IgG (horseradish peroxidase-linked) and an enhanced chemiluminescence (ECL) detection system were obtained from Amersham (Arlington Heights, IL). All other chemicals were of the highest purity commercially available.

**Cell Culture.** Bovine aortic EC (repository no. AG07680B) were obtained from the National Institute of Ageing Cell Culture Repository, Coriell Institute for Medical Research (Camden, NJ). These cells tested positive for the EC-specific von Willebrand factor and angiotensin-1-converting enzyme activity. They tested negative for α smooth muscle actin. EC were maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, plus bovine serum albumin as a standard.

**Cell Viability.** In ethanol-treated cells, cell viability, as described below, between passage 12–15. In ethanol-treated cells, after treatment for 10 min with 0.125% trypsin-EDTA at 37°C. EC dinucleotide (800 Ci/mmol) was obtained from New England Nuclear (Levitzki and Bar-Sinai, 1991; Helmreich and Hofmann, 1996). Recent evidence suggests that inhibitory G proteins (Gi1,2,3), which are pertussis toxin (PTX)-sensitive, and Goq may play a role in both agonist- and shear stress-induced activation of endothelial nitric oxide synthase (eNOS) in EC (Cooke et al., 1991; Ohno et al., 1993). Interestingly, in the liver and central nervous system ethanol-induced alterations in the abundance and function of G proteins coupled to effectors such as adenylyl cyclase and phospholipase C have been demonstrated (Hoffman and Tabakoff, 1990; Iles and Nagy, 1995).

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(Na⁺ form). The L[14C]citrulline was eluted with 2 × 0.5 ml of distilled water that was collected and counted by liquid scintillation spectrometry. Basal eNOS activity varied considerably between different EC batches. For this reason, experimental groups were always treated and compared in parallel.

**PTX-Catalyzed ADP-Ribosylation.** G protein substrates of PTX were assayed using PTX-catalyzed incorporation of [32P]ADP-ribose from [32P]NAD, as described previously (Cahill et al., 1994). Membranes (40–60 μg) were resuspended in 100 μl of 100 mM Tris-HCl (pH 8.0) containing 5 mM DTT, 10 mM thymidine, 6 mM MgCl₂, 2 mM GTP, 2.5 mM ATP, and 10 μM [32P]NAD. PTX (100 μg/ml) was activated by prior incubation in 50 mM HEPES (pH 8.0), containing 20 mM DTT, 0.125% SDS, and 0.1 mg/ml BSA for 30 min at room temperature. After addition of activated PTX (20 μg/ml), the membrane preparations were incubated for 90 min at 30°C. The ADP-ribosylation reactions were stopped by centrifugation at 15,000g for 3 min and the pellet was resuspended in SDS-sample buffer containing 62.5 mM Tris-HCl (pH 6.8), 2% SDS, 10% (v/v) glycerol, and 5% (v/v) β-mercaptoethanol. The samples were boiled for 10 min before being resolved on a 10% SDS-polyacrylamide gel. Gels were dried on cellophane and exposed to Kodak XAR-5 film with an intensifying screen at −70°C for 1 to 3 days.

**Western Blotting.** Membrane proteins (15–40 μg/lane) were separated on 10% SDS-polyacrylamide gel as described previously (Cahill et al., 1994). After SDS-polyacrylamide gel electrophoresis, the separated proteins were electrophoretically transferred to nitrocellulose membranes (Hybond-C, Amersham, Arlington Heights, IL) using a Transphor electroblotter unit (Hoefer Scientific Instruments, San Francisco, CA) at 100 V for 2 h. After transfer, the membranes were incubated for 2 h in blocking solution containing 50 mM Tris base (pH 7.6), 4 mM MgCl₂, and 140 mM NaCl (TBS) supplemented with 5% nonfat dry milk, 0.1% Tween 20, and 2 mg/ml sodium azide. The membranes were then washed three times for 5 min each with TBS containing 0.1% (v/v) Tween 20 and 2% (v/v) Nonidet P-40. The membranes were incubated with the specific antisera in TBS Tween 20, 0.02% (w/v), for 1 h at room temperature with gentle rocking. After washing the blots three times for 10 min they were incubated with the secondary antibody solution (horse-radish peroxidase conjugated) and diluted 1:5000 in TBS-Tween 20 for 40 min at room temperature with gentle agitation. The blots were finally washed three times for 10 min each before they were processed using the ECL detection system (Amersham), as described by the manufacturer. Blots were then covered in cellophane and exposed to Hyperfilm-ECL (Amersham) for 15 to 30 s. Equal protein loading was confirmed by India-ink staining of protein in each lane of the same blot. The signal intensity (integral volume) of the appropriate bands on the autoradiogram was analyzed using a Personal Densitometer (Molecular Dynamics, Sunnyvale, CA) and the Imagequant software package (Biosoft, Indianapolis, IN).

**Statistics.** The data shown are the mean ± S.E.M. Statistical significance was estimated using the following analysis: unpaired Student’s t test for comparison of two groups; ANOVA followed by the Bonferroni/Dunnett’s test for multiple comparison; Wilcoxon-Signed Rank test for the densitometric data. A probability value of p < .05 was considered significant.

**Results.**

**Effect of Ethanol on eNOS under “Static” Conditions.** The effect of ethanol on basal eNOS activity in bovine aortic EC cultured under “no flow” (static) conditions was first determined. EC were exposed to ethanol (0–160 mM) for 2 h before eNOS activity was determined as described in Experimental Procedures. Ethanol dose-dependently increased basal eNOS activity with significant enhancement evident at 0.8 mM and a maximum response was achieved at 40 mM (Fig. 2a); 2.06 ± 0.17-fold increase (n = 11) for 40 mM ethanol, which was used in subsequent experiments. Ethanol significantly increased eNOS activity in the absence of any effect on eNOS protein levels, either after 2- or 48-h exposure (Fig. 2b). Moreover, ethanol (2- and 24-h exposure) did not induce iNOS protein expression in these cells (Fig. 2c). Ethanol concentration in petri dishes, with or without EC, was constant over the experimental time (2 h) used (data not shown), suggesting that neither significant evaporation nor metabolism of ethanol was occurring. Ethanol, at the concentrations used, had no significant effect on EC viability as assessed by trypan blue exclusion (data not shown).
Although PTX pretreatment (100 ng/ml, 24 h) had no significant effect on basal eNOS activity in static EC, it significantly attenuated the ethanol (40 mM)-induced increase in basal eNOS activity (Fig. 3). This suggests that ethanol is mediating its stimulatory effect on eNOS by a mechanism involving, in part, a PTX-sensitive G protein. Ethanol did not significantly alter EC G protein levels as determined by Western blot analysis; EtOH 40 mM, 24 h: 97.8 ± 3.0%, 95.1 ± 3.9%, and 96.3 ± 1.7% of control expression for Gi3, Ga-a1–2, and Ga-q respectively.

Effect of Ethanol on Laminar Flow-Stimulated eNOS. EC in 100-mm culture dishes were exposed to steady laminar flow/shear stress by being placed on an orbital shaker at various rotational frequencies at 37°C. There was a laminar flow- and time-dependent increase in eNOS activity (data not shown) with a significant response obtained at 100 rpm (shear stress ~2.78 dynes/cm²) for 30 min (static: 0.32 ± 0.03, 100 rpm: 0.85 ± 0.2 pmol [14C]citrulline/mg/min, n = 3, P < .05). The effect of ethanol on laminar flow-induced eNOS activity was determined by pretreating EC in the absence or presence of ethanol (40 mM) for 2 h before exposing them to laminar flow (30 min, 100 rpm). Ethanol pretreatment significantly enhanced the laminar flow-induced eNOS response from 0.62 ± 0.1 to 1.06 ± 0.06 pmol [14C]citrulline/mg/min, p < .05 (Fig. 4). PTX treatment, (100 ng/ml, 24 h), significantly attenuated both the laminar flow-
Effect of Laminar Flow and Ethanol on PTX-Catalyzed ADP-Dependent Ribosylation of Gia Substrates.

To determine whether laminar flow- and ethanol-induced increases in eNOS activity correlated with changes in G protein functional activity, we measured PTX-catalyzed NAD-dependent ADP ribosylation of Gia substrates in these EC. PTX catalyzed the incorporation of \([^{32}\text{P}]\)ADP-ribose into one major peptide band (~40 kDa) in cultured EC, a protein that comigrated with Gia proteins immunodetected using specific antibodies (data not shown). The ribosylation was linear over a range of 10 to 100 \(\mu\)g of membrane protein. PTX-catalyzed ribosylation of Gia substrates was significantly increased in laminar flow-exposed EC compared with static controls (Fig. 5). In addition, PTX-catalyzed ribosylation of Gia substrates was significantly increased in ethanol-treated cells under both static and laminar flow conditions when compared with respective controls (Fig. 5).

Effect of Ethanol on Pulsatile Flow-Stimulated eNOS.

EC in transcapillary cultures were exposed to “low” and “high” pulsatile flow in the absence or presence of ethanol as described in Experimental Procedures. EC exposed to high flow (35 ml/min, shear stress ~12 dynes/cm\(^2\)) exhibited a significantly increase compared with low flow (2 ml/min, shear stress ~0.5 dynes/cm\(^2\)) in eNOS activity from 0.52 ±0.02 to 0.73 ±0.01 pmol \([^{14}\text{C}]\)citrulline/mg/min (Fig. 6a). PTX treatment inhibited the pulsatile flow-induced increase in eNOS activity (Fig. 6a). Under low-flow conditions EC treated with ethanol (40 mM) had significantly greater eNOS activity than control cells; 0.76 ±0.03 versus 0.48 ±0.04 pmol \([^{14}\text{C}]\)citrulline/mg/min (Fig. 6b). PTX inhibited this potentiation by ethanol (Fig. 6c). Ethanol did not further potentiate the high-flow-induced increase in eNOS activity (Fig. 6b).

Discussion

The current studies define for the first time a stimulatory effect of ethanol on basal and flow-induced eNOS activity that is mediated in part by a mechanism involving a PTX-sensitive G protein. Although ethanol consumption can result in higher death rates from several diseases including certain cancers and cirrhosis, beneficial effects have been implied with respect to coronary artery disease (CAD; Friedman and Kimball, 1986; Klatsky et al., 1992; Doll et al., 1994). Our results demonstrate that treatment of cultured EC with ethanol under “static” conditions increased eNOS activity in a dose-dependent manner. Moreover, in EC exposed to the dynamic effects of the ubiquitous stimulus of flow (physiologic range of shear stress: 0–30 dynes/cm\(^2\)) ethanol enhanced eNOS activity after exposure to low shear stresses (0.5 and 2.78 dynes/cm\(^2\)). The enhanced eNOS activity was independent of a change in eNOS or iNOS protein expression but dependent on activation of an inhibitory guanine nucleotide binding protein (Gi protein) inasmuch as ethanol treatment increased PTX-catalyzed ribosylation of Gia substrates and PTX treatment inhibited eNOS activity in static and flow-stimulated cells after ethanol treatment.

Several previous studies have examined the acute and chronic effects of ethanol treatment on cardiovascular function. Acute ethanol exposure causes vasoconstriction (Altura and Altura, 1982) whereas chronic exposure results in the development of tolerance to its vasoconstrictive effect and the vasoconstrictive effects of other agonists, most notably phenylephrine (Strickland and Woolies, 1988), an effect that requires an intact endothelium (Kynch et al., 1984). Moreover, although chronic ethanol consumption can eventually lead to endothelial or vascular smooth muscle cell dysfunction resulting in the loss of endothelium-dependent tolerance and the development of hypertension, there is compelling evidence to suggest that moderate ethanol consumption (1–4 drinks per day) can be beneficial by reducing the incidence of CAD and myocardial infarction (Friedman and Kimball, 1986; Klatsky et al., 1992, Maclure, 1993; Doll et al., 1994). However, the underlying mechanism of this protective effect is at present unknown. A blood alcohol level of 0.1 g%, the legal limit in many states, is approximately equivalent to 25 mM ethanol. Several previous studies have examined the effect of ethanol on cell biology at nonphysiological concentrations, i.e., in excess of 100 mM. However, the range of...
ethanol concentrations used in this study was 0.8 to 160 mM. Although the majority of experiments were performed with 40 mM ethanol, significant effects on basal eNOS activity were demonstrated at as low as 0.8 mM ethanol.

The mechanism by which ethanol activates a PTX-sensitive G protein and subsequently stimulates eNOS activity under static and flow conditions is at present unclear. Davda et al. (1993) demonstrated that ethanol enhanced eNOS activity in response to agonists such as bradykinin, ionomycin, and ATP without affecting basal enzyme activity. Although the mechanisms involved in this process were not addressed, the temporal relationship of ethanol's potentiation of ATP- and ionomycin-stimulation of eNOS activity suggested that alterations in agonist-receptor interactions or postreceptor signaling events may contribute to the effect of ethanol on eNOS activity (Davda et al., 1993). In this regard, several studies have reported ethanol-induced changes in intracellular signaling mechanisms in various cell types. In brain and hepatocytes ethanol potentiates signaling events through a PTX-sensitive G protein-dependent pathway (Nagy and Desilva, 1992; Wand et al., 1993). Several studies have demonstrated that eNOS activation and subsequent NO production is regulated at the level of a PTX-sensitive G protein in cultured EC (Flavahan and Vanhoutte, 1990; Cushing et al., 1990). Moreover, activation of inhibitory G proteins by several endothelial receptor agonists can result in endothelium-dependent NO-induced relaxation (Flavahan and Vanhoutte, 1990). In addition, direct activation of Gia proteins with mastoporan or GTPγS can mimic this effect (Flavahan and Vanhoutte, 1990; Hou et al., 1997) and shear stress-induced stimulation of eNOS activity is dependent on activation of inhibitory G proteins (Ohno et al., 1993). Under flow conditions, ethanol could be acting to enhance the signaling of a shear stress-coupling mechanism. Indeed, our data demonstrate that ethanol increases the functional activity of a PTX-sensitive G protein both under static and flow conditions, a signaling pathway that has previously been shown to couple shear stress-sensitive responses to eNOS stimulation. There was also a PTX-insensitive component of the EtOH response representing ~30% in both static and flow cultures. This suggests that other mechanism(s) may also be involved, e.g., other G proteins such as Gq. However, we did not observe any significant change in Gαq protein levels after

![Fig. 6](image)

**Fig. 6.** The effect of pulsatile flow on EC eNOS activity. A, NOS activity was determined in EC exposed to low (2 ml/min, 0.5 dyne/cm²) and high (35 ml/min, 12 dyne/cm²) pulsatile flow in the absence (open columns) or presence (hatched columns) of PTX (100 ng/ml). B, effect of ethanol on pulsatile flow-stimulated eNOS activity. NOS activity was determined in EC exposed to low and high pulsatile flow, in the absence (open columns) or presence (closed columns) of ethanol (EtOH, 40 mM) as detailed in Experimental Procedures and Fig. 1. C, NOS activity was determined in EC after exposure to low pulsatile flow (2 ml/min, 0.5 dyne/cm²) in the absence (open columns) or presence (closed columns) of ethanol (40 mM) with (+PTX) or without (-PTX) PTX pretreatment. Data are mean ± S.E.M., n = 3. *P < .05.

![Fig. 7](image)

**Fig. 7.** PTX-catalyzed [32P]-ADP ribosylation of Gia substrates in EC, pretreated with (EtOH) or without (Control) ethanol (40 mM, 2 h) under low or high pulsatile flow conditions. A representative autoradiograph is shown (top) with the cumulative densitometric data of three separate experiments. *P < .05 versus low flow control.
Ethanol treatment. In addition, because our experiments were performed in serum-containing media, it is possible that ethanol could be activating a mitogen receptor coupled to a PTX-sensitive G protein. However, this seems unlikely because similar results were obtained using serum-depleted media (data not shown).

Several animal models of hypercholesterolemia and atherosclerosis suggest that the etiology of the endothelial dysfunction and reduced eNOS response is in part due to decreased expression/activity of an inhibitory Gi protein (Flavahan and Vanhoutte, 1990; Freeman et al., 1996). Our data suggest that in EC under static and steady flow conditions, ethanol treatment increased the functional activity of Giα proteins such that inactivation of these proteins with PTX inhibited the ethanol-induced increase in eNOS activity. Although ethanol pretreatment significantly increased eNOS activity in cells exposed to submaximal levels of shear stress (steady laminar: 2.78 dynes/cm²; low pulsatile flow: 0.5 dynes/cm²) it did not further enhance the high pulsatile flow (12 dynes/cm²)-induced increase in eNOS activity, suggesting that flow and ethanol share similar mechanisms for eNOS stimulation, namely, activation of a PTX-sensitive G protein.

The mechanisms coupling flow forces to EC responses are not yet fully understood. Most studies have focused on the cellular responses to direct mechanical stresses, which appear to involve an interplay between structural elements and biochemical second messengers (Davies and Tripathi, 1993). Cell surface proteins and extracellular matrix, linked by transmembrane proteins to the cytoskeleton, activate ion channels and enzymes by mechanical deformation. A change in either the extracellular concentration of bioactive ligands at the cell surface or nutrient exchange across the cell membrane as a result of fluid movement may also be an indirect mechanism of mechanotransduction. However, there is much evidence to suggest that fluid shear stress is the principal stimulus of activation of eNOS. Because shear stress is a function of fluid velocity and viscosity it is possible to change the shear stress to which EC are subjected by changing either fluid flow rate (velocity) or fluid viscosity. In this manner, investigators have demonstrated that flow-dependent changes in eNOS are shear stress-dependent and not merely flow rate-dependent by comparing responses at a set flow rate but different fluid viscosities (Davies and Tripathi, 1993). These data therefore diminish the possibility that the changes in NOS activity are due to changes in the exchange of nutrients across the cell membrane. Regardless of the precise mechanism(s) involved, our data confirm the reported stimulatory effect of flow on endothelial NOS activity and demonstrate an ethanol enhancement of that response.

Both animal and human studies have revealed the importance of NO regulation in coronary arteries under normal conditions and after coronary artery dysfunction (Moncada et al., 1991; Freeman et al., 1996). Impairment of coronary artery flow is an important feature of ischemia-reperfusion and is known to be associated with a significant diminution of basal and stimulated release of NO (Ma et al., 1993). Moreover, there is reduced eNOS activity in the human atherosclerotic epicardial and microvascular coronary circulation such that agonist-induced coronary dilation is compromised (Quyumi et al., 1997). Under normal conditions, basal release of NO plays a crucial role in the maintenance of basal coronary artery flow and appears to be essential for sustaining mechanical activity and protecting against platelet aggregation and extracellular matrix production (Moncada et al., 1991).

Although the prevailing theory supported by a number of clinical and animal studies indicates that ethanol’s ability to elevate serum high-density lipoprotein cholesterol levels is an important mechanism in ameliorating CAD (Hojnacki et al., 1988), other mechanisms whereby ethanol could exert its beneficial effect on CAD have evolved: namely, its ability to inhibit thrombosis by decreasing platelet aggregation (Renaud et al., 1992), its ability to increase fibrinolytic activity by increasing plasminogen activators (Ridker et al., 1994), and its ability to suppress the immune response (Watson et al., 1994). It is noteworthy that NO has been shown by several laboratories to mediate these same responses both in vitro and in vivo (Moncada et al., 1991). However, further in vivo experiments using NOS inhibitors will be required to determine whether ethanol-induced changes in platelet aggregation, fibrinolytic activity, and immunosuppression are mediated in part by NO and contribute to the apparent beneficial effect of ethanol on CAD.

In summary, we have demonstrated for the first time that ethanol enhances eNOS activity in cultured EC under static and flow conditions via a mechanism involving a PTX-sensitive G protein. Because of the critical role endothelial-derived NO plays in modulating vascular function and structure, it is tempting to speculate that the beneficial effects of alcohol consumption are mediated, at least in part, by ethanol-induced stimulation of eNOS activity.

References


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