Activation of Guanine Nucleotide-binding Proteins and Induction of Endothelial Tissue-type Plasminogen Activator Gene Transcription by Alcohol*

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The mechanism by which moderate alcohol ingestion lowers the risk of cardiovascular disease is unknown but may be due, in part, to the ability of alcohol to increase the level of tissue-type plasminogen activator (t-PA). Human endothelial cells were treated with low concentrations of ethanol (0.25–25 mM, 0–24 h), which are associated with moderate alcohol consumption. Although treatment with ethanol alone did not affect t-PA gene transcription or mRNA expression, it augmented isoproterenol (ISO)-stimulated t-PA gene transcription and mRNA levels by 3.4- and 2.8-fold, respectively, and decreased plasminogen activator inhibitor-1 mRNA levels by 3.4- and 2.8-fold, respectively, and mRNA levels by 3.4- and 2.8-fold, respectively, and decreased plasminogen activator inhibitor-1 mRNA levels by 65%. These effects of ethanol correlated with 2.5- and 6.9-fold increases in ISO-stimulated cyclic AMP levels and 4x-cyclic AMP response element heterologous promoter activity, respectively. To determine whether alcohol-induced changes in agonist-stimulated cyclic AMP levels were because of modulation of guanine nucleotide-binding proteins (G-proteins), we assessed the effects of ethanol on Ga and Ga. Although ethanol did not affect the expression of Ga or Ga, it increased ISO-stimulated Ga GTPase and GTP binding activity by 2.2- and 2.9-fold and decreased UK14304-stimulated Ga GTPase and GTP binding activity by 38 and 80%. These results indicate that treatment with relatively low concentrations of ethanol enhances agonist-stimulated cyclic AMP-dependent t-PA gene transcription in vascular endothelial cells through differential modulation of G protein.

Epidemiological studies suggest that moderate alcohol ingestion (i.e. 1–2 drinks/day) reduces the risk of cardiovascular-related disease (1, 2). Although the precise mechanism(s) by which alcohol protects against atherosclerotic heart disease is not known, recent studies suggest that some of the beneficial effects of alcohol may be mediated by its ability to prevent vascular thrombosis and occlusion (3). Because the rupture of atherosclerotic plaques with ensuing thrombosis is widely believed to be the predominant etiology underlying acute coronary syndromes (4), endogenous mediators such as the proteolytic enzyme, tissue-type plasminogen activator (t-PA),1 and its inhibitor, plasminogen activator inhibitor (PAI-1) are important in regulating the thrombotic process (5). The function of t-PA is to convert plasminogen to a proteolytic enzyme, plasmin, which digests fibrin-dependent blood clots. Thus, thrombotic vascular complications may be attenuated by agents that increase the secretion of t-PA and/or decrease the expression of PAI-1 (6–8). Indeed, the plasma level of t-PA has been shown to be inversely correlated with the risk of myocardial infarction (9).

The induction of t-PA gene transcription is regulated, in part, by cAMP and transcription factors that bind to activator protein-2 and shear-stress response element (10–12). The ability of cAMP to mediate increases in t-PA expression has been demonstrated in many cell types including hepatocytes, fibroblasts, and endothelial cells (13–16). Although higher concentrations of alcohol (i.e. >100 mM) have been shown to modulate guanine nucleotide-binding protein (G-protein) activity and cAMP levels in neuronal tissues or cell lines (17–19), it is not known whether lower concentrations of alcohol (i.e. <5 mM) have similar effects in vascular endothelial cells and whether these effects, if present, translate into specific gene expression that contributes to the cardiovascular protective effects of moderate alcohol ingestion. Thus, the purpose of this study is to characterize the effects of alcohol on G-protein function in vascular endothelial cells and to determine whether concentrations of alcohol that are associated with the ingestion of less than two alcoholic beverages can up-regulate t-PA expression via G-protein-mediated cAMP-dependent signaling pathways.

EXPERIMENTAL PROCEDURES

Materials—All standard culture reagents were obtained from JRH Bioscience. Isoproterenol, propranolol, ascorbic acid, creatinine phosphate, phosphocreatine kinase, GTP-8, phenylmethylsulfonyl fluoride, leupeptin, aprotinin, dithiothreitol, bovine serum albumin, ATP, GDP, GTP, 8-bromo-cAMP, and cholera and pertussis toxins were purchased from Sigma. UK14304 (α2-adrenergic receptor agonist) was a gift from Pfizer. The radioisotopes, [γ-32P]GTP (30 Ci/μmol), [35S]GTP (1250 Ci/μmol), [α-32P]UTP (800 Ci/μmol), and [α-32P]CTP (3000 Ci/μmol) and the polyclonal rabbit antiserum to Ga (RM/1) were supplied by NEN Life Science Products. The polyclonal rabbit antiserum P4 was raised against a purified decapeptide corresponding to the COOH-terminal regions of Ga (20). Protein molecular weight markers were purchased from Life Technologies, Inc. The chemiluminescence detection kit (ECL) was obtained from Amersham Pharmacia Biotech. The polyvinylidene difluoride transfer membrane (pore size 0.2 μm) was purchased from Bio-Rad. The heterologous 4x-CRE promoter luciferase reporter construct (p.CRE-Luc) was purchased from Stratagene (San Diego, CA). The full-length human t-PA and PAI-1 cDNAs were obtained from...
Activation of Goα and Alcohol-induced t-PA Expression

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Cell Culture—Endothelial cells were harvested from human saphenous veins and human and bovine aortas as described (21, 22). The cells were cultured at 37 °C in a growth medium containing Dulbecco’s modified Eagle’s medium (supplemented with 1% antibiotic (Life Technologies, Inc.), 10% fetal calf serum (Hyclone), and an antibiotic mixture of penicillin (100 units/ml), streptomycin (100 mg/ml), Fungi- zone (250 mg/ml). Relatively pure endothelial cell cultures were con- firmed by Nomarski optical microscopy (Olympus IX70, 40 × objective) and by immunofluorescence staining with anti-Factor VIII antibodies. All passages were performed with a disposable cell scraper (Costar), and by immunofluorescence staining with anti-Factor VIII antibodies. Zone (250 ng/ml). Relatively pure endothelial cell cultures were con- firmed by Nomarski optical microscopy (Olympus IX70, 40 × objective) and by immunofluorescence staining with anti-Factor VIII antibodies.

Western Blotting—Partially purified membranes were prepared from control and ethanol-treated endothelial cells as described previously (22). Membrane proteins (25 μg) and molecular weight markers were separated by SDS/polyacrylamide gel electrophoresis (10% running, 4% stacking gel). The proteins were electrophoretically transferred onto polyvinylidene difluoride membranes and incubated overnight at 4 °C with blocking solution (5% nonfat dry milk and 0.1% Tween 20 in phosphate-buffered saline) before to the addition of the following dilu- tions were added to the mixture:

- Tris-HCl (25 mM, pH 7.4), leupeptin (10 μM), sodium phosphate (50 mM), NaCl (100 mM), Triton X-100 (1%), and SDS (0.1%).
- The polyvinylidene difluoride membranes were then washed twice with phosphate-buffered saline buffer containing 0.1% Tween 20 and then treated with donkey anti-rabbit horseradish peroxidase anti- body (1:4000) (Amersham Pharmacia Biotech). Radiographic chemiluminescence was performed several times at 23 °C, and the appropriate exposures were subjected to densitometric analysis.

Nuclear Run-on Assay—Confluent endothelial cells (5 × 105 cells) were treated with ethanol (2.5 mM, 12 h) in the presence or absence of isoproterenol (100 nM). Nuclei were isolated, and in vitro transcription was performed with [α-32P]UTP (800 Ci/mmol), as described previously (24).

Northern Blotting—Equal amounts of total RNA (20 μg) were sepa- rated by 1.2% formaldehyde-agarose gel electrophoresis, transferred overnight onto Hybond nylon membranes (Amersham Pharmacia Biotech) by capillary action, and baked for 2 h at 80 °C before prehybridization. Radiolabeling of full-length human t-PA or PAI-1 cDNA were performed using random hexamer priming, [35S]GTP, and Klenow (Amersham Pharmacia Biotech). The membranes were hybridized with the indicated probes overnight at 45 °C in a solution containing 50% formamide, 5× SSC (1× SSC = 0.15 M NaCl and 0.015 M sodium citrate), 2.5× Denhardt’s solution, 25 mM sodium phosphate buffer (pH 6.5), 0.1% SDS, and 250 μg/ml salmon sperm DNA. All Northern blots were subjected to stringent washing conditions (0.2× SSC, 0.1% SDS) at 65 °C before autoradiography with intensifying screen at −80 °C for 24 h. Equalization of RNA loading was assessed by re-probing all blots with human β-tubulin (ATCC number 37556).

GTPase Assay—Membranes (30 μg) from endothelial cells treated with the indicated conditions were incubated for 90 min at 22 °C in the presence or absence of specific COOH-terminal antisera before GTPase assay as described (23). Preliminary studies revealed that maximal inhibition of receptor-G-protein coupling was achieved by the antisera to the triple tandem 4–5′-CRE motif (20, 24). Equal amounts of purified, denatured full-length human t-PA, human, β-tubulin (ATCC n number 37855), and linearized pGEM-3z cDNA were vacuum-transferred onto nitrocellulose membranes using a slot blot apparatus (Schleicher & Schuell). Hybridization of radiolabeled mRNA transcripts to the nitrocellulose membranes was carried out at 45 °C for 1 h in a buffer containing 50% formamide, 5× SSC, 2.5× Denhardt’s solution, 25 mM sodium phosphate buffer (pH 6.5), 0.1% SDS, and 250 μg/ml salmon sperm DNA. The membranes were then washed with 1× SSC, 0.1% SDS for 1 h at 65 °C before autoradiography for 22 h at −80 °C. Band intensities were subjected to analyses by laser densitometry.

Data Analysis—Band intensities from Northern, Western, and nu- clear run-on assays were analyzed densitometrically by the Na- tional Institutes of Health Image Program (25). All values are expressed as mean ± S.E. compared with controls and among separate experiments. Paired and unpaired Student’s t tests were employed to determine the significance of changes in GTPase, GTP binding, and luciferase activities. A significant difference was taken for p values less than 0.05.

RESULTS

Cell Culture—There were no observable adverse effects of ethanol, isoproterenol, and 8-bromo-cAMP on cell number, morphology, or immunofluorescent staining for Factor VIII-related antigens. Cellular confluence and viability as deter- mined by trypan blue exclusion were maintained for all treatment conditions described.

Effects of Ethanol on t-PA and PAI-1 mRNA Expression—Treatment of human vascular endothelial cells with ethanol (0–2.5 mM, 24 h) alone did not affect steady-state t-PA mRNA expression. However, at an ethanol concentration of 25 mM, there was a 36 ± 5% reduction in t-PA mRNA levels compared with basal levels (p < 0.05, n = 3). Stimulation of untreated endothelial cells with isoproterenol (100 nM) in- creased t-PA mRNA levels by 48 ± 6% (p < 0.05 compared with base line, n = 3). Endothelial cells treated with 0.25 and 2.5 mM

2 h at 4 °C, and the precipitate was collected by centrifugation at 12,000 × g for 10 min. Preliminary studies indicated that all α2 and α1 were completely precipitated by this procedure because Western blot analysis of the supernatant with the P4 and RM/1 antisera did not reveal the presence of 40–41- or 46–52-kDa proteins, respectively. The precipitates were then resuspended in a buffer containing HEPES (50 mM, pH 7.4), NaF (100 mM), sodium phosphate (50 mM), NaCl (100 mM), Triton X-100 (1%), and SDS (0.1%). The final pellet containing the immunoprecipitated [35S]GTP-γ-S-labeled G-protein was counted in a liquid scintillation counter (LS 1800, Beckman Instruments, Inc., Ful- lerton, CA). Nonspecific activity was determined in the presence of excess unlabeled GTP(γ)S (100 mM).

cAMP Assay—Confluent endothelial cells were treated for 12 h with the indicated concentrations of ethanol. After the addition of isobutyryl- methoxanethione (0.5 mM) for 15 min, cells were stimulated with isoproterenol (100 nM) in the presence or absence of propranolol (100 μM) for another 15 min. Unstimulated cells were treated with isobutyryl- methoxanethione for 30 min. Cells were scraped on ice, pelleted, and resus- pended in ice-cold isobutylmethylxanthine (0.5 mM), booted for 3 min, and frozen at −70 °C. The intracellular cAMP level was determined using a radioimmunoassay kit with [3H](cAMP (Amersham Pharmacia Biotech).
ethanol showed a 2.8- and 2.3-fold increase in isoproterenol-stimulated t-PA mRNA levels compared with ethanol alone (p < 0.05 for both, n = 3). At an ethanol concentration of 25 mM, there was no significant increase in isoproterenol-stimulated t-PA mRNA expression compared with ethanol alone (p > 0.05, n = 3). Similar results were observed with human aortic endothelial cells (data not shown). These results indicate that low concentrations of ethanol (< 10 mM) augment isoproterenol-stimulated t-PA expression in vascular endothelial cells.

Treatment of endothelial cells with increasing concentrations of ethanol (0–25 mM, 24 h) also did not significantly affect PAI-1 steady-state mRNA levels (Fig. 1B). However, stimulation with isoproterenol (100 mM) alone decreased PAI-1 mRNA levels by 28 ± 5% (p < 0.05, n = 3). Ethanol concentrations of 0.25 and 2.5 mM decreased PAI-1 mRNA levels by 40 ± 5 and 65 ± 6%, respectively (p < 0.05 for both, n = 3) in the presence of isoproterenol. Interestingly, higher concentrations of ethanol (i.e. 25 mM) produced no further decrease in PAI-1 mRNA levels in the presence of isoproterenol compared with that of isoproterenol alone (32 ± 4% versus 28 ± 5%, p > 0.05, n = 3). The effects of ethanol on t-PA mRNA expression are almost completely the opposite effects of ethanol on PAI-1 expression. These findings suggest that the effects of low concentrations of ethanol favor fibrinolysis by increasing the ratio of t-PA to PAI-1 levels.

**Effects of Ethanol on t-PA Gene Transcription**—Nuclear run-on assays showed that ethanol (2.5 mM, 12 h) alone did not affect t-PA gene transcription relative to that of β-tubulin (Fig. 2). Stimulation of untreated endothelial cells with isoproterenol (100 mM) increased t-PA gene transcription by 2.2-fold relative to that of β-tubulin (p < 0.05, n = 2). In endothelial cells treated with ethanol (2.5 mM) and stimulated with isoproterenol (100 mM), t-PA gene transcription was increased by 3.4-fold compared with untreated control cells (p < 0.1) and increased by 2.8-fold compared with ethanol treatment alone (p < 0.05). These findings are consistent with changes in t-PA mRNA expression and suggest that low concentrations of alcohol augment isoproterenol-stimulated t-PA gene transcription but, by itself, may have little, if any, effects.

Preliminary studies using different amounts of radiolabeled RNA transcripts demonstrate that under our experimental conditions, hybridization was linear and nonsaturable. The density of each t-PA band was standardized to the density of its corresponding β-tubulin band (relative intensity). The specific intensity of each band was determined by the lack of hybridization to the nonspecific pGEM cDNA vector.

**Effect of Ethanol on G-protein Expression**—Because isoproterenol stimulated an increase in t-PA gene transcription and mRNA expression is dependent upon coupling to specific G-proteins, particularly Go alpha, we investigated whether low concentrations of alcohol affect G-protein expression. Treatment of endothelial cells with ethanol (0–50 mM, 12 h) did not affect the protein levels of Go alpha or Go gamma (Fig. 3). Furthermore, stimulation of endothelial cells with the β2-adrenergic agonist, isoproterenol (100 nM), or the α2-adrenergic agonist, UK14304 (10 nM), had no effect on Go alpha and Go gamma expression, respectively (data not shown).

The P4 (Go alpha) and RM/1 (Go gamma) antisera were relatively specific because recognition of their respective α subunits could be blocked in the presence of excess decapeptides from which they were derived (20, 22). In addition, we found that the amount of Go alpha1 and common β subunit as determined by the QL and SWI antisera, respectively, was also unaffected by treatment with ethanol (data not shown).

**Effect of Ethanol on Agonist-stimulated GTPase Activity**—The basal GTPase activity in human endothelial cell membrane was 2.5 ± 0.5 pmol/min/mg. Treatment with ethanol (0–25 mM, 12 h) had no effect on basal GTPase activity (Fig. 4A). Stimulation of untreated endothelial cells (control) with isoproterenol (100 nM) and UK14304 (10 nM) increased GTPase activity by 2.6- and 5.4-fold, respectively (6.5 ± 1.1 and 13.4 ± 0.7 pmol/min/mg, p < 0.01 compared with basal levels, n = 4). Compared with isoproterenol-stimulated untreated control cells, ethanol-treated endothelial cells showed a progressive increase in isoproterenol-stimulated GTPase activity, resulting in a maximal 2.2-fold increase in isoproterenol-stimulated GTPase activity at an ethanol concentration of 2.5 mM (6.5 ± 1.1 to 14.5 ± 1.2 pmol/min/mg, p < 0.01, n = 4). In contrast, ethanol-treated endothelial cells showed a maximal 38 ± 5% decrease in UK14304-stimulated GTPase activity at an ethanol concentration of 2.5 mM (13.4 ± 0.7 to 8.3 ± 1.1 pmol/min/mg, p < 0.05, n = 4). Interestingly, higher ethanol concentrations (i.e. 25 mM) resulted in no further increase or decrease in isoproterenol- and UK14304-stimulated GTPase activity, respectively (11.7 ± 1.8 and 10.0 ± 2.1 pmol/min/mg, respectively, p > 0.05 for both, compared with GTPase values at ethanol concentration of 2.5 mM). In a time-dependent manner, ethanol (2.5 mM) increased isoproterenol-stimulated GTPase activity by 290 ± 22% and decreased UK14304-stimulated GTPase activity by 42 ± 7%, with maximal effects of both agonists occurring after 12 h of ethanol exposure (Fig. 4B).
isoproterenol- and UK14304-stimulated GTPase activity, respectively (p < 0.05 for both), compared with basal unstimulated GTPase activity of 2.5 ± 0.5 pmol/min/mg (Fig. 4C). In contrast, pretreatment with the P4 antisera abolished <10% of isoproterenol-stimulated GTPase activity, whereas the RM/1 antisera decreased <5% of UK14304-stimulated GTPase activity. Furthermore, isoproterenol- and UK14304-stimulated GTPase activity were completely blocked with propranolol (0.1 mM) and rauwolscine (1 μM), respectively (data not shown).

Effects of Ethanol on Agonist-stimulated GTPαS-Binding Activity—To determine the effects of ethanol on specific G-protein α subunit GTP-binding activity, αi and αi2, from ethanol-treated membranes were radioisotopically labeled with [35S]GTPαS and immunoprecipitated with specific antisera directed against αi and αi2 subunits. Treatment with ethanol (0–25 mM, 12 h) alone did not significantly affect basal αi and αi2 GTP binding activity (p > 0.05 for both). Endothelial cells stimulated with isoproterenol (100 nM) and UK14304 (10 nM) have αi and αi2 GTP binding activity of 2.0 ± 0.3 and 17.1 ± 1.3 fmol/min/mg, respectively (Fig. 5). Treatment with ethanol (0–25 mM, 12 h) caused a progressive increase in αi GTP binding activity with a maximal 2.9-fold increase (5.9 ± 0.7 fmol/min/mg, p < 0.05, n = 3) occurring at an ethanol concentration of 2.5 mM. In contrast, the same membrane from ethanol-treated cells exhibited a progressive decrease in αi2 GTP binding activity with a maximal 80 ± 5% decrease (3.5 ± 0.3 fmol/min/mg, p < 0.01, n = 3), also occurring at an ethanol concentration of 2.5 mM.

Effects of Ethanol on Adenylyl Cyclase Activity—To determine whether the effects of ethanol on Gαi and Gαi2 correlate with downstream effector activity (i.e. adenylyl cyclase), we measured intracellular cAMP levels in ethanol-treated endothelial cells. Treatment with ethanol (0–25 mM, 12 h) did not affect basal cAMP levels (Fig. 5). Stimulation with isoproterenol (100 nM) increased cAMP level from 2.7 ± 0.6 pmol/500,000 cells (p < 0.05, n = 4) (Fig. 6). This increase in isoproterenol-stimulated cAMP level was completely blocked with propranolol (2.9 ± 0.4 pmol/500,000 cells, p > 0.05 compared with basal levels).

Endothelial cells treated with ethanol (2.5 mM, 12 h) showed a significant increase in isoproterenol-stimulated cAMP level (2.7 ± 0.2 to 8.2 ± 0.5 pmol/500,000 cells, p < 0.05). This increase represented a 2.5-fold increase in absolute isoproterenol-stimulated cAMP level (2.2 ± 0.2 to 5.5 ± 0.3 pmol/500,000 cells, p < 0.05). Interestingly, lower concentrations of ethanol (0.25 mM, 12 h) did not produce a significant increase in isoproterenol-stimulated cAMP level compared with untreated cells, and higher concentrations of ethanol (25 mM, 12 h) did not produce further increases in isoproterenol-stimulated cAMP level compared with cells treated with 2.5 mM ethanol. These findings are consistent with the effects of alcohol on t-PA exp-
pression and suggest that low concentrations of alcohol augment isoproterenol-stimulated cAMP-dependent t-PA gene transcription.

Effects of Ethanol on cAMP-dependent Promoter Activity—
The cAMP-dependent gene transcription involves transcription factors that bind to the cAMP response element (CRE) of target genes (26). To determine whether increases in cAMP levels produced by low concentrations of ethanol are sufficient to transactivate promoters containing CRE, we transfected endothelial cells with a heterologous luciferase reporter construct containing four tandem CREs (p.CRE-Luc). Treatment of transfected endothelial cells with isoproterenol (100 nM, 12 h) or 8-bromo-cAMP (10 μM, 12 h) increased p.CRE-Luc activity by 3.7- and 3.3-fold, respectively (p < 0.05 for both, n = 3) (data not shown).

Treatment of transfected endothelial cells with ethanol (0–50 mM, 12 h) alone did not affect basal p.CRE-Luc activity. However, stimulation of ethanol-treated transfected cells with isoproterenol (100 mM) augmented p.CRE-Luc activity compared with that of isoproterenol alone (Fig. 7A). The maximal isoproterenol-stimulated p.CRE-Luc activity in ethanol-treated cells was 6.9- and 2.1-fold higher than that of untreated and isoproterenol-stimulated control cells, respectively, and occurred at an ethanol concentration of 2.5 mM. Higher concentrations of ethanol (25–50 mM, 12 h) resulted in reductions in isoproterenol-stimulated p.CRE-Luc activity. In a time-dependent manner, ethanol (2.5 mM) augmented isoproterenol-stimulated p.CRE-Luc activity compared with that of untreated endothelial cells (5.6-fold versus 3.5-fold, respectively, p < 0.05) (Fig. 7B). The increase in isoproterenol-stimulated p.CRE-Luc activity occurred after 12 h of ethanol exposure, and this time point is consistent with the maximal increase in isoproterenol-stimulated Gαs GTPase activity.

DISCUSSION

We have shown that alcohol concentrations corresponding to moderate ingestion (i.e. <10 mM) augment agonist-stimulated endothelial t-PA gene transcription via G-protein-mediated cAMP-dependent pathway. Furthermore, these effects of alcohol were associated with a reciprocal decrease in agonist-stimulated PAI-1 expression. Higher concentrations of alcohol (≥25 mM), however, did not produce further increases in intracellular cAMP levels, but instead, decreased isoproterenol-stimulated GTPase activity and t-PA expression and increased PAI-1 expression. Interestingly, treatment with alcohol alone did not affect basal G-protein activity, intracellular cAMP level, p.CRE-Luc activity, or t-PA gene transcription and expression. These findings are in contrast to previous studies showing that alcohol decreased Gαs expression and adenylyl cyclase activity in brain tissues (17, 27). However, these previous studies, higher concentrations of alcohol were used for a much longer duration. Our results, therefore, indicate that alcohol at concentrations associated with moderate ingestion augments β2-adrenergic receptor-stimulated intracellular cAMP level and t-PA gene transcription.

The effect of alcohol on isoproterenol-stimulated Gαs activity is rather specific because alcohol concomitantly down-regulated UK14304-stimulated GTPase activity. Because the α2-adrenergic receptor agonist, UK14304, is predominantly coupled to Gα12 in vascular endothelial cells (28), these results suggest that alcohol differentially modulates Gαs and Gα12 activity. Because there were no observable changes in the amounts of Gαs and Gα12, our results also indicate that alcohol modulates G-protein function rather than expression. Specificity in receptor-G-protein coupling was confirmed by [35S]GTPγS binding of specific G-protein α subunits and with studies using carboxyl-terminal-directed antibodies to Gαs and Gα12 that specifically blocked isoproterenol- and UK14304-stimulated GTPase activity, respectively. Thus, the net effects of alcohol on Gαs and Gα12 activity is to favor receptor-mediated activation of adenylyl cyclase. However, there was no direct stimulatory effect of alcohol on adenylyl cyclase activity, because alcohol alone was unable to increase intracellular cAMP level.
The regulation of gene transcription by cAMP is mediated by trans-acting factors, which bind to CRE (5'-ATGAGCTCAT-3') of target genes (26). Although the consensus sequence for CRE is not present in the t-PA promoter, a functional CRE-like element has been identified that acts synergistically with a putative activator protein-2 binding site to induced t-PA gene transcription by phorbol 12-myristate 13-acetate (29). Indeed, previous studies have shown that cholera toxin and dibutyryl-cAMP can directly induce t-PA gene transcription in rat hepatocytes (13), although in HeLa cells, the activation of cAMP-dependent transcription factor(s) alone is not sufficient to transactivate the t-PA promoter (14, 29). These studies, therefore, suggest that other cis-acting element(s) may play an important role in transactivating the t-PA promoter. Interestingly, a recent study by Grenett et al. (30) showed that alcohol alone can increase t-PA gene transcription. Although we did not observe an increase in t-PA gene transcription and expression with alcohol alone, there are subtle differences between the two studies. In contrast to the previous study, our study utilized lower concentrations of alcohol (i.e. 2.5 mM) for a longer duration (i.e. up to 24 h). Furthermore, our finding that alcohol alone does not increase t-PA gene transcription is consistent with the other findings of this study showing that alcohol alone does not stimulate Gaα activity, cAMP production, or CRE promoter activity.

The precise mechanism by which alcohol alters membrane signal transduction and increases t-PA expression is not known. Previous studies have shown that high concentrations of alcohol (i.e. 100 mM) increase α2-adrenergic and muscarinic receptor expression in a neuronal NG108–15 cells (31). Although we cannot exclude the possibility that alcohol may have induced similar changes in β2-adrenergic receptor affinity and density in our study, these effects of alcohol, however, are unlikely given the relatively low concentrations of alcohol used and the lack of significant changes in the EC50 (~1 nM) of isoproterenol with respect to pCRE-Luc activity in alcohol-treated cells. It is also possible that with longer duration, alcohol, even at low concentrations, may ultimately induce changes in the level of Gaα and Gaα2. Indeed, chronic alcohol treatment, albeit at high concentrations, have been shown to inhibit adenylyl cyclase activity via increasing Gaα and decreasing Gaα expression in neuronal cells (17, 32). Under the conditions of our study, however, the observed changes in isoproterenol-stimulated G-protein activity, cAMP levels, and t-PA gene expression occurred in the absence of significant changes in endothelial Gaα and Gaα2 expression.

The etiology of acute coronary syndromes is thought to be because of plaque rupture with ensuing vascular thrombosis and occlusion (4). The degree of vascular thrombosis and thrombolysis is regulated, in part, by the level of t-PA and PAI-1. Recent epidemiology studies suggest that moderate alcohol consumption (i.e. 1–2 drinks/day) is associated with high serum t-PA levels and lower incidence of myocardial infarctions (1, 2, 9). Because the ingestion of two glasses of wine (an alcoholic content of 4%) represents an alcohol concentration of approximately 7 mM in vivo (33), our findings suggest that alcohol may lower the risk of cardiovascular disease by augmenting t-PA level and inhibiting PAI-1 expression. Both of these effects of alcohol would enhance the fibrinolytic activity in the vessel wall.

In summary, we have provided a potential mechanism by which low concentrations of alcohol may protect against atherosclerotic heart disease. Because alcohol alone does not induce, but rather, augments agonist-stimulated t-PA gene transcription via Gaα-mediated cAMP-dependent pathway, we speculate that moderate alcohol consumption may be more beneficial in terms of t-PA production in active individuals with frequent elevations in catecholamine levels. Consequently, β-blocker therapy, which is beneficial in cardiac ischemia, may paradoxically diminish some of the antithrombotic effects of alcohol on the vessel wall. We propose that low but not high concentrations of alcohol up-regulate agonist-stimulated cAMP and t-PA expression through differential effects on Gaα and Gaα activity. It remains to be determined, however, the mechanism by which alcohol modulates the activities of specific G proteins.

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