Dose-dependent Activation of Antiapoptotic and Proapoptotic Pathways by Ethanol Treatment in Human Vascular Endothelial Cells

DIFFERENTIAL INVOLVEMENT OF ADENOSINE*

Jie Liu, Zhigang Tian‡, Bin Gao, and George Kunos§

From the Laboratory of Physiologic Studies, National Institute on Alcohol Abuse and Alcoholism, Bethesda, Maryland 20892

Moderate but not heavy drinking has been found to have a protective effect against cardiovascular morbidity. We investigated the effects of ethanol (EtOH) treatment on the cell survival-promoting phosphatidylinositol 3-kinase (PI3K)/Akt pathway in cultured human umbilical vein endothelial cells (HUVEC). Exposure of cells to 2–20 mM EtOH resulted in rapid (<10 min) induction of Akt phosphorylation that could be prevented by pertussis toxin or the PI3K inhibitors wortmannin and LY294002. Among the downstream effectors of PI3K/Akt, p70S6 kinase, glycogen synthase kinase 3β (GSK3β), and IκB-α were phosphorylated, the latter resulting in 3-fold activation of NF-κB. EtOH also activated p44/42 mitogen-activated protein kinase in a PI3K-dependent manner. Low concentrations of EtOH increased endothelial nitric-oxide synthase activity, which could be blocked by transfection of HUVEC with dominant-negative Akt, implicating the PI3K/Akt pathway in this effect. The adenosine A1 receptor antagonist 1,3-dipropylocyclopentylxanthine prevented the phosphorylation of Akt observed in the presence of EtOH, adenosine, or the A1 agonist N6-cyclpentyladenosine. Incubation of HUVEC with 50–100 mM EtOH resulted in mitochondrial permeability transition and caspase-3 activation followed by apoptosis, as documented by DNA fragmentation and TUNEL assays. EtOH-induced apoptosis was unaffected by DPCPX and was potentiated by wortmannin and LY294002. We conclude that treatment with low concentrations of EtOH activates the cell survival promoting PI3K/Akt pathway in endothelial cells by an adenosine receptor-dependent mechanism and activation of the proapoptotic caspase pathway by higher concentrations of EtOH via an adenosine-independent mechanism can mask or counteract such effects.

Alcohol has been the most widely used and misused drug throughout human history. Aside from its well known neurobehavioral effects, alcohol also influences cardiovascular variables. The ingestion of one or two alcohol-containing drinks has acute effects on heart rate, blood pressure, cardiac output, myocardial contractility, and regional blood flow, actions that are generally not clinically important. During the last few decades, moderate alcohol consumption has been associated with a reduced risk for ischemic cardiovascular disease (2, 3), whereas chronic heavy drinking was found to increase cardiovascular morbidity and mortality (3). Although the cardioprotective effect of moderate drinking is generally attributed to an elevation of high density lipoproteins and reduced platelet aggregation (4), alcohol has been shown to alter endothelial function (5); such changes may also contribute to its cardioprotective effects. Vascular endothelial cells, because of their location at the interface of blood and the vessel wall, are susceptible to the influence of various blood-borne agents. The endothelium is now recognized as an important regulator of vascular tone, and the controlled proliferation of vascular endothelial cells is a key step in angiogenesis. In the present study we examined the hypothesis that ethanol may influence both pro- and antiapoptotic pathways in the vascular endothelium in ways that could contribute to its dose-dependent effects on cardiovascular morbidity.

The serine/threonine protein kinase Akt was originally discovered as the cellular homolog (c-Akt) of the transforming retrovirus AKT8 (6). It is a novel kinase with similarities to both protein kinase C and protein kinase A, so it is also referred to as protein kinase B (7). Akt is a downstream mediator of phosphatidylinositol 3-kinase (PI3K), recruited to the plasma membrane by binding the lipid products of PI3K, phosphatidylinositol 3,4-bisphosphate and 3,4,5-trisphosphate, which bind to the pleckstrin homology domain present in a number of cytosolic signaling proteins including Akt (8). Akt is a multifunctional mediator of PI3K-dependent signaling and functions to promote cell survival as well as the transcription and translation of proteins involved in cell cycle progression (9). Receptor-induced activation of Akt is blocked by PI3K inhibitors and by the expression of dominant-negative forms of PI3K (10–17).

Phosphorylated Akt activates different downstream effectors, including endothelial nitric-oxide synthase (eNOS), an enzyme involved in vascular remodeling and angiogenesis and also responsible for maintaining systemic blood pressure (18,

The abbreviations used are: PI3K, phosphatidylinositol 3-kinase; MAP, mitogen-activated protein; GSK, glycogen synthase kinase; eNOS, endothelial nitric-oxide synthase; PTX, pertussis toxin; CPA, Nγ-cyclpentyladenosine; DPCPX, 1,3-dipropylocyclopentylxanthine; GTPγS, guanosine 5′-3-O-(thio)triphosphate; MPT, mitochondrial permeability transition; TUNEL, terminal deoxynucleotidyltransferase-mediated dUTP nick end-labeling; PARP, poly(ADP-ribose) polymerase; HUVEC, human umbilical vein endothelial cells; PBS, phosphate-buffered saline; ROS, reactive oxygen species; DiOC6, 3,3′-dihexyloxocarbocyanine.

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ On leave from the Institute of Immunology, University of Science and Technology of China, Hefei 230027, China.

§ To whom correspondence should be addressed: National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, 12420 Parklawn Dr., Rm. 120, MSC-8115, Bethesda, MD 20892-8115. Tel.: 301-443-2069; Fax: 301-480-0257; E-mail: gkunos@mail.nih.gov.

This paper is available on line at http://www.jbc.org
Another major downstream effector is p70S6 kinase, which phosphorylates the ribosomal S6 protein in response to stimulation with mitogens and preferentially increases the translation of mRNAs containing 5'-terminal oligopyrimidine tracts (14, 20). Glycogen synthase kinase 3 (GSK-3) is also a target of Akt (13, 21–25). Inhibition of GSK-3 by Akt inhibits apoptosis and promotes cell survival (22, 26). Akt is well established as a mediator of cell survival, as its activation protects against apoptosis induced by withdrawal of growth factor or serum (27). NF-κB is a downstream mediator of Akt in anti-apoptotic signaling (28). When bound to its cytosolic inhibitor, IkB, NF-κB is sequestered in the cytoplasm. Upon its phosphorylation by IkB kinases, IkB is degraded, which allows NF-κB to move to the nucleus and activate the transcription of anti-apoptotic proteins (29).

Here we report that in the presence of low concentrations (2–20 μM) of ethanol, the cell survival-promoting PI3K/Akt pathway and several of its downstream effectors, including eNOS, GSK-3, p70S6 kinase, and NF-κB, are activated via an adenosine-dependent mechanism, whereas at concentrations of 50–100 μM, ethanol promotes serum deprivation-induced endothelial cell apoptosis independently of adenosine byactivating caspase-3 and its downstream target, PARP-1. These dose-dependent, opposing effects on endothelial cell survival pathways may contribute to the bimodal effects of ethanol consumption on cardiovascular morbidity.

**Experimental Procedures**

**Materials**—Ethanol, pertussis toxin (PTX), GF 109203X, wortmannin, and LY294002, Nα-cyclopentyladenosine (CPA), 1,3-dipropylcyclopentylxanthine (DPCPX), and adenosine were purchased from Sigma. Rabbit anti-human antibodies to native and phosphorylated forms of Akt (Ser-473; catalog No. 9272 and 9271), Leb (α-catalase No. 9242 and 9246), GSK-3α/β (Ser-21; catalog No. 9331), p70S6 kinase (catalog No. 9205), p44/42 MAP kinase (catalog No. 9102 and 9101), and caspase-3 (catalog No. 9662) were purchased from New England Biolabs (Beverly, MA). Anti-poly(ADP-ribose) polymerase (PARP; catalog No. SA-253) and caspase-3 cellular activity assay kits were purchased from Biomol (Plymouth Meeting, PA). Anti-rabbit IgG (horseradish peroxidase-linked) and enhanced chemiluminescence reagent (ECL) detection system were obtained from PerkinElmer Life Sciences. [3H]GTPyS (catalog No. 9262) was purchased from PerkinElmer Life Sciences, and [α-32P]dATP was obtained from Amersham Biosciences. TdT recombinant was purchased from Invitrogen.

**Cell Culture**—Primary cultured human umbilical vein endothelial cells were purchased from ATCC. The plastic culture flask was precoted with 0.2% gelatin for at least 1 h prior to seeding the cells. HUVEC were maintained in EBM-2 medium (Clonetics) supplemented with 2% fetal bovine serum and growth factors such as hydrocortisone, hFGF-B, VEGF, R1-IGF-1, ascorbic acid, hEGF, GA-1000, and heparin plus 100 units/ml penicillin and 100 μg/ml streptomycin in a humidified atmosphere of 5% CO2 at 37°C. All experiments were performed using HUVEC at 2–4 passages, with no cells being used beyond the fourth passage. For serum starvation, the regular medium was removed and replaced with medium 199.

**Western Blot Analyses**—Western immunoblotting was done as previously described (30). HUVEC cells were cultured in serum-free medium overnight, prior to the addition of ethanol, to reduce the baseline levels of Akt phosphorylation. Cell lysate protein (60 μg) from an equal number of cells was size-fractionated by 10% SDS-PAGE and then transferred electrophoretically to a nitrocellulose membrane. The membrane was blocked by incubation for 2 h in TBPS buffer (1× PBS, 0.1% Tween 20) containing 5% (w/v) nonfat dry milk at room temperature and then immunoblotted with primary antibody (at 1:1000 dilution in TBPS plus 3% bovine serum albumin) overnight at 4°C and horseradish peroxidase-conjugated secondary antibody (1:2000) in blocking buffer for 2 h at room temperature. Immunoreactive bands were visualized using an ECL detection system.

**[3H]GTPyS Binding**—Confluent HUVEC were rinsed twice in PBS and harvested by scraping. The cells were then homogenized in TME-Na buffer (50 mM Tris-HCl, 3 mM MgCl2, 0.2 mM EGTA, 100 mM NaCl, pH 7.4), and the homogenate was centrifuged at 48,000 × g for 20 min at 4°C. The pellet was resuspended in TME-Na, homogenized, and adjusted to a protein concentration of 1 μg/μl with TME-Na. The membrane homogenate was then used for measuring agonist-stimulated [3H]GTPyS binding according to Griffin et al. (31). HUVEC membranes (100 μg) were incubated in TME-Na buffer containing 0.1 mg/ml bovine serum albumin with 30 μM GDP, 0.1 nM [3H]GTPyS, and different concentrations of cAMP-dependent glass tubes with or without 100 ng/ml PTX or 100 nM DPCPX. The total assay volume was 0.5 ml, and triplicate aliquots were incubated at 30°C for 1 h. The reaction was terminated by the addition of 2 ml of ice-cold wash buffer (50 mM Tris-HCl, pH 7.4) followed by vacuum filtration through glass fiber (type B) filters. Filters were rinsed three more times with wash buffer, air dried, and retained radioactivity was determined by liquid scintillation spectrometry. Basal binding was assayed in the absence of ethanol and in the presence of GDP.

**Transfections and Fluorescent Cell Sorting**—Plasmids pEGFP-N1 (CLONTECH) and dominant-negative Akt were co-transfected into HUVEC P2 cells. Using an initial seeding density of 1 × 104 cells/cm2, HUVEC were ready for transfection at 18–24 h post-seeding. For each 75-cm2 flask of cells to be transfected, 30 μl of Lipofectin and 5 μg of each DNA were diluted separately in 500 μl of Opti-MEM I reduced serum medium. Solutions were combined, gently mixed, and incubated for 45 min at room temperature to allow formation of DNA-lipid complexes. The normal medium was replaced with 8 ml of Opti-MEM I reduced medium, and the DNA-lipid complexes were added subcellularly. The flask was incubated for 45 min at room temperature. The transfection mixture was replaced with basal media containing supplements. Transfected cells were identified by cell sorting at 18–24 h post-transfection, and only the positive cells were used for the eNOS activity assay.

**eNOS Activity**—eNOS activity was measured by monitoring the conversion of [1-14C]Harginine to 1-[14C]Hcitrulline (eNOSelect Assay Kit, Stratagene). Cells were trypsinized and washed with PBS and lysed in an adequate volume of homogenization buffer (25 mM Tris-HCl, pH 7.4, 1 mM EDTA, 1 mM EGTA). The supernatant was separated from the homogenate, and protein concentration was adjusted to 5 μg/ml. The reaction mixture was prepared on ice by adding the following components: 25 μl of 2 reaction buffer (50 mM Tris-HCl, pH 7.4, 6 μM BH4, 2 μM flavin adenine dinucleotide, 2 μM flavin adenine mononucleotide), 5 μl of 10 mM NADPH (freshly prepared in 10 mM Tris-HCl, pH 7.4), 1 μl of [1-14C]Harginine (50 μCi/ml), 5 μl of 6 μM CaCl2, and 4 μl of H2O. The reaction was initiated by the addition of 10 μl of protein extract (total volume of 50 μl) and carried out for 30 min at 37°C. Rat cerebellum extract and Nα-nitro-1-arginine methyl ester HCl were used as positive and negative control. This reaction was terminated by the addition of 400 μl of stop buffer containing 50 mM HEPES, pH 5.5, and 5 mM EDTA. The reaction mixture was then passed through a provided resin. The [14C]Hcitrulline generated was quantified by liquid scintillation spectrometry.

**Luciferase Reporter Assay**—The NF-κB luciferase promoter construct was purchased from Stratagene. Transient transfection was performed as described above. The transfection mixture was incubated with medium M199 overnight. Cells were stimulated for another 8 h with ethanol and then washed twice with PBS and lysed in 200 μl (for 12-well culture plate) of 1× passive lysis buffer. Cell lysates were centrifuged for 30 s at top speed in a refrigerated microcentrifuge, the cleared lysates were transferred to a fresh tube, and protein content was determined. Equal amounts of protein were used for reporter enzyme analyses using the Luciferase Reporter Assay System (Promega) and a luminometer.

**Flow Cytometric Analysis of Apoptosis by Measurement of Mitochondrial Transmembrane Potential (ΔΨm) and the Generation of Reactive Oxygen Species (ROS)—ΔΨm and ROS generation were measured according to (32). HUVEC were treated with or without ethanol for 6 h under standard conditions and harvested by trypsinization. ΔΨm was measured directly by using 40 mM 3,3'-diethyloxycarbocyanine (DiOC6 (3), Molecular Probes, Eugene, OR). Fluorescence was measured after staining the cells for 15 min at 37°C. To assess ROS generation by flow cytometry, cells were treated with 2 μM hydroethidine (Molecular Probes) for 15 min at 37°C. The probes were excited with a laser at 488 nm, and ROS generation was measured through a 530/30 nm (DiOC6 (3)) or a 575/56 nm (ethidium) bandpass filter. Logarithmic amplification was used to detect the fluorescence of the probes.

**DNA Fragmentation Assay**—Following treatment, DNA was isolated using a DNA Isolation Kit (Genta, Minneapolis, MN). Briefly, 300 μl of cell lysates were incubated with 1.5 μl of RNase A solution at 37°C for 5 min. 100 μl of protein precipitation solution was added to each sample, and after centrifugation at 13,000 × g for 1 min, the superna
EtOH Activates Pathways Involved in Endothelial Cell Survival

RESULTS

**Ethanol Induces Phosphorylation of Akt in HUVEC**—A blood alcohol level of 0.1%, the legal limit for driving in many states, is equivalent to ~22 mM ethanol. The ethanol concentrations used in this study were 10 µM–100 mM, which spans the range of behaviorally sub-threshold to severe intoxicating levels. Ethanol treatment caused strong, concentration-dependent activation of Akt in HUVEC (Fig. 1A), activation being evident in the presence of as little as 10 µM ethanol. The time course is illustrated in Fig. 1B, with activation beginning at 5 min after the addition of ethanol and peaking at 30 min. Furthermore, the time- and concentration-dependent activation of Akt was evident both in the absence (upper panels) and in the presence of serum and growth factors (lower panels). Additionally, activation of Akt following ethanol treatment was completely antagonized by two structurally dissimilar inhibitors of PI3K, wortmannin and LY294002 (Fig. 1C); the ethanol metabolite acetaldehyde was inactive in this pathway (Fig. 1D).

**Ethanol Induces MAP Kinase Activation in a PI3K-dependent Mechanism**—Since the p44/42 MAP kinase can be activated by PI3K (33), we tested whether ethanol treatment can activate MAP kinase in HUVEC. As illustrated in Fig. 2A, treatment with 20 mM ethanol results in rapid phosphorylation of MAP kinase within 5 min, an effect that is abrogated in the presence of the PI3K inhibitors wortmannin or LY294002 (Fig. 2B).

**Ethanol Induces Akt Activation by a PTX-sensitive G Protein-dependent Mechanism**—To explore the mechanism by which ethanol treatment induces PI3K/Akt activation, we examined the effect of ethanol on GTP-S binding in HUVEC membranes. As shown in Fig. 3A, in the presence of 2–100 mM ethanol GTP-S binding in HUVEC membranes was increased by up to 25%. This effect was largely inhibited by PTX, a G protein inhibitor, or by DPCPX, an A1 adenosine receptor inhibitor. This indicates the possible involvement of G<sub>αi/o</sub> protein(s) and A1 adenosine receptors in the activation of Akt phosphorylation by ethanol treatment. The mechanisms involved in Akt phosphorylation were further examined by Western blot analyses. As shown in Fig. 3B, Akt phosphorylation in response to ethanol treatment was largely inhibited by PTX but not by GF 109203X, a protein kinase C inhibitor.

**Activation of Downstream Effectors of Akt by Ethanol Treatment**—We next investigated ethanol activation of pathways downstream of Akt. As shown in Fig. 4A, p70S6 kinase, GSK-3β, and Erk-B can all be phosphorylated by treatment with 10 mM ethanol. Further experiments with transfected NF-κB promoter/luciferase constructs indicated NF-κB promoter activity was increased by up to 3-fold in the presence of 2–20 mM ethanol, but with higher concentration of ethanol (50 mM) the activity declined (Fig. 4B). Similar results have been obtained for eNOS activity (Fig. 5). The increase in eNOS activity was dependent on ethanol concentration and on the duration of ethanol exposure. A time course study showed that eNOS activity peaked at 10 min following ethanol exposure (Fig. 5A). eNOS activity in HUVEC was increased by treatment with 20 mM ethanol, but activity declined toward basal levels with increasing ethanol concentrations (Fig. 5B). This ethanol-induced increase in eNOS activity could be blocked by transfection of HUVEC cells with dominant-negative Akt, implicating the PI3K/Akt pathway in eNOS activation by ethanol (Fig. 5C).
phosphorylation caused by ethanol, adenosine, or CPA, which implicates adenosine in this effect of ethanol.

**High Concentrations of Ethanol Induce Apoptosis Independently of Adenosine**—Fetal alcohol syndrome induced by maternal drinking has been linked to apoptotic cell death in the central nervous system. Therefore, we tested whether treatment with high concentrations of alcohol can also lead to apoptosis in vascular endothelial cells. Because there is increasing evidence that altered mitochondrial function is linked to apoptosis (40, 41), we first checked the ability of ethanol treatment to induce a mitochondrial permeability transition (MPT) state as an apoptotic early event, by measuring $\Delta \psi_m$ and ROS generation. We measured $\Delta \psi_m$ by monitoring the incorporation of the fluorescent probe DiOC$_6$. After 18 h, 20 mM ethanol was added for another 30 min. Cell extracts were then prepared and subjected to Western blot analysis using anti-phospho-Akt and anti-Akt antibodies.

In 76% of the cells, a low $\psi_m$ is associated with altered mitochondrial function, we also determined whether exposure to 50 mM ethanol potentiated mitochondrial $O_2$ generation by the oxidative conversion of hydroethidine to ethidium. We noted that 5% of the cells exhibited an increase in ethidium fluorescence. As illustrated in Fig. 7, discrete cell populations shifted from the right lower quadrant to the left lower and left upper quadrants. Taken together, treatment with 50 mM ethanol not only resulted in a loss of $\psi_m$ but also led to uncoupling of oxidative metabolism and to the generation of ROS in HUVEC cells as early as only 6 h of incubation. No significant changes in fluorescence have been noted in the presence of...
lower concentration (2–20 mM) of ethanol (data not shown).

We next analyzed DNA fragmentation following exposure of HUVEC to ethanol and wortmannin (Fig. 8A). Incubation of HUVEC under conditions of serum and growth factor deprivation for 24 h caused a minority of the cells to undergo apoptosis as verified by a slight increase in DNA fragmentation compared with control. Exposure of serum-deprived HUVEC to 2–10 mM of ethanol did not significantly alter the levels of DNA fragmentation (Fig. 8A) and cell apoptosis (Fig. 8B). However, cells exposed to 50 mM ethanol displayed a dramatic increase in DNA fragmentation (Fig. 8A) and cell apoptosis (Fig. 8B). Moreover, incubation of serum-deprived HUVEC with 100 nM wortmannin, a PI3K inhibitor, also caused an increase in DNA fragmentation without influencing the ethanol-induced increase in cell apoptosis (Fig. 8A) and increased cell apoptosis (Fig. 8B). This indicates that the PI3K/Akt pathway has a protective effect on cell survival. Finally, the selective adenosine A1 receptor antagonist DPCPX did not block 50 mM ethanol-induced apoptosis. Similar results were obtained by measuring the activity of caspase-3, an executor of apoptosis that has been shown to be implicated in apoptosis in mammalian cells. As shown in Fig. 9A, serum starvation caused an increase in caspase-3 activity, which was not affected by 2 mM ethanol but was markedly potentiated by 20–50 mM ethanol treatment. Consistent evidence was obtained by immunoblotting, showing a significantly increased level of the active 17-kDa subunit of caspase-3 and increased cleavage of the 85-kDa PARP (Fig. 9B) in serum-deprived HUVEC exposed to 20–50 mM ethanol.

**DISCUSSION**

The present findings document a bimodal action of ethanol on pathways involved in endothelial cell survival. At concentrations up to 20 mM that do not cause significant intoxication in vivo, ethanol was found to activate the PI3K/Akt survival pathway in cultured human vascular endothelial cells. Consistent with this activation, several key downstream effectors of Akt known to be linked to anti-apoptotic, cell survival-promoting effects were also activated. In contrast, at concentrations of 50–100 mM, which cause severe intoxication in vivo, ethanol activated the pro-apoptotic caspase-3/PARP-1 pathway and apoptosis, which is analogous to its pro-apoptotic effect in the central nervous system. To best interpret these findings, we propose a model in Fig. 10 that may partly explain the bimodal action of ethanol in the cardiovascular system.

In this model, treatment with low concentrations (<20 mM) of ethanol elicits the activation of Akt and its downstream effectors including NF-kB and eNOS. Some of these effectors, such as the p70S6 kinase, can also be activated by MAP kinase (33), and we found that ethanol rapidly activates MAP kinase in a PI3K-dependent manner (Fig. 2). It has been clearly shown that activation of Akt, eNOS, and NF-kB play an important role in promoting angiogenesis and inhibiting apoptosis in HUVEC (27–29, 45). Importantly, the effects of ethanol treatment on Akt phosphorylation were evident not only in serum-starved cells but also in the presence of physiological levels of serum and growth factors, which suggests that similar changes are likely present in the intact organism. Thus, activation of Akt by low concentrations of ethanol likely promotes endothelial cell survival and thus may contribute to the protective effects...
Several mechanisms have been implicated in the cardioprotective effect of moderate alcohol drinking. These include modulation of blood lipoproteins (55), reduced platelet activation and thrombosis (55), and activation of e protein kinase C (56). Inhibition of the synthesis of the vasoconstrictor peptide endothelin-1 by components of red wine may account for the added benefits accrued from drinking red wine (57).

The results of the present study suggest that, in addition to these mechanisms, ethanol induction of Akt activation may also contribute to the cardiovascular protective effect of moderate alcohol drinking. Akt phosphorylation results in the activation of a number of downstream effectors implicated in cell survival and also in the inhibition of apoptotic pathways, as suggested by the finding that the PI3K inhibitor wortmannin potentiates apoptosis in HUVEC. Together, these effects could promote angiogenesis induced by moderate hypoxia and would thus contribute to the protective effects of moderate drinking in ischemic heart disease.

REFERENCES

1. Kunos, G., and Varga, K. (1996) NIAAA Res. Monogr. 31, 243–261
2. Tansese, M., Hu, F. B., Willett, W. C., Stampfer, M. J., and Rimm, E. B. (2001) J. Am. Coll. Cardiol. 38, 1836–1842
3. Van Tol, A., and Hendriks, H. F. (2001) Curr. Opin. Lipidol. 12, 19–23
4. Constanti, J. (1997) Coron. Artery Dis. 8, 645–649
5. Davila, R. K., Chandler, L. J., Crews, F. T., and Guzman, N. J. (1993) Hypertension 21, 939–945
6. Staal, S. P. (1997) Proc. Natl. Acad. Sci. U. S. A. 84, 5034–5037
7. Jones, P. F., Jakubowicz, T., Fittogi, F. J., Maurer, F., and Hemmings, B. A. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 4171–4175
8. Bottomley, M. J., Salim, K., and Panayotou, G. (1998) Biochem. Biophys. Acta 1396, 165–183
9. Vanhaesebroeck, B., and Alessi, D. R. (2000) Biochem. J. 346, 561–576
10. Franke, T. F., Yang, S. I., Chan, T. O., Datta, K., Kazlauskas, A., Morrison, D. K., Kaplan, D. R., and Tsichlis, P. N. (1995) Cell 81, 725–736
11. Kohn, A. D., Kovacina, K. S., and Roth, R. A. (1995) EMBO J. 14, 4288–4295
12. Alessi, D. R., Andjelkovic, M., Caudwell, B., Cron, P., Morrive, N., Cohen, P., and Hemmings, B. A. (1996) EMBO J. 15, 6541–6551
13. Crespo, D. E., Alessi, D. R., Cohen, P., Andjelkovic, M., and Hemmings, B. A. (1995) Nature 378, 785–789
14. Burgering, B. M. T., and Coffer, P. J. (1995) Nature 372, 599–602
15. del Peso, L., Gonzalez-Garcia, M., Page, C., Herrera, R., and Nunez, G. (1997) Science 278, 687–689
16. Reif, K., Burgering, B. M. T., and Cantrell, D. A. (1997) J. Biol. Chem. 272, 14426–14433
17. Scheid, M. P., and Derijndorff, B. M. T. (1997) J. Biol. Chem. 272, 1906–1913
18. Dimmeler, S., Fleming, I., Fisslthaler, B., Hermann, C., Busse, R., and Zeiher, A. M. (1999) Circulation 100, 1030–1036
19. van Veen, P. C., de Brouw, K. M. T., de Vries Smits, A. M. M., van Lintel, J., Bijl, W., van de Laar, M., and Burgering, B. M. T. (1998) J. Biol. Chem. 273, 13150–13156
20. Gold, R. M., Scheid, M. P., Santos, L., Law-Lawson, M., Roth, R. A., Matsuishi, L., Derijndorff, V., and Krebs, D. L. (1999) J. Biol. Chem. 274, 18949–19005
21. Madge, L. A., and Pober, J. S. (2000) J. Biol. Chem. 275, 15458–15465
22. Bismashov, J. A., and Makarov, S. S. (1999) Nature 401, 86–90
23. Foo, S. Y., and Nol, G. P. (1999) Trends Cell Biol. 5, 225–235
24. Liu, J., Gao, B., Mariah, S., Sanyal, A. J., Khanolkar, A. D., Makriyannis, A., and Kunos, G. (2000) Biochem. J. 346, 835–840
25. Griffin, G., Tan, Q., and Aboud, M. E. (2000) J. Pharmacol. Exp. Ther. 292, 886–894
26. Guo, T. L., Miller, M. A., Datar, S., Shapiro, I. M., and Shenker, B. J. (1998) Toxicol. Appl. Pharm. 152, 397–405
27. Toker, A. (2000) Mol. Pharmacol. 57, 652–658
28. Acvioleti, C. C., Huamanchano, A., Perez, E., Rojas, S., Bravo, I., and Vazquez, J. (1997) Pharmacol. Rev. 49, 837–392
29. Fredholm, B. B., and Wallman-Johnsson, A. (1996) Pharmacol. Toxicol. 78, 120–123
30. Kresss, S. W., Ghirnikar, R. B., Diamond, I., and Gordon, A. S. (1993) Mol. Pharmacol. 44, 1021–1026
31. Nagy, L., I. Diamond, I., and Gordon, A. S. (1991) Mol. Pharmacol. 40, 812–817
32. Grechak, R., and Dickenson, J. M. (2000) Br. J. Pharmacol. 130, 867–874
33. Takasuga, S., Kadoya, T., Kitahara, S., and Haze, O. (1999) J. Biol. Chem. 274, 19545–19550
34. Higuchi, H., Adachi, M., Miura, S., Gores, G. J., and Ishii, H. (2001) Hepatology 33, 1442–1443
EtOH Activates Pathways Involved in Endothelial Cell Survival

34, 320–328
41. de la Monte, S. M., and Wands, J. R. (2001) Alcohol. Clin. Exp. Res. 25, 889–897
42. Kroemer, G. (1997) Nat. Med. 3, 614–620
43. Marchetti, P., Hirsch, T., Zamzami, N., Castedo, M., Decaudin, D., Susin, S. A., Masse, B., and Kroemer, G. (1996) J. Immunol. 157, 4830–4836
44. Kroemer, G., Petit, P., Zamzami, N., Vayssiere, J., and Mignotte, B. (1995) FASEB J. 9, 1277–1287
45. Datta, S. R., Brunet, A., and Greenberg, M. E. (1999) Genes Dev. 13, 2965–2977
46. Bouma, M. G., van den Wildenberg, F. A., and Buurman, W. A. (1996) Am. J. Physiol. 276, C322–C329
47. Linden, J. (2001) Annu. Rev. Pharmacol. Toxicol. 41, 775–787
48. Jarvis, M. F., and Williams, M. (1988) Pharmacol. Biochem. Behav. 30, 707–714
49. Sapru, M. K., Diamond, I., and Gordon, A. S. (1994) J. Pharmacol. Exp. Ther. 271, 542–548
50. Nagy, L. E., Diamond, I., Casso, D. J., Franklin, C., and Gordon, A. S. (1996) J. Biol. Chem. 265, 1946–1951
51. Diao, L., and Dunwiddie, T. V. (1996) J. Pharmacol. Exp. Ther. 278, 542–546
52. Green, D. R., and Reed, J. C. (1998) Science 281, 1309–1312
53. Liu, X., Kim, C. N., Yang, J., Jemmerson, R., and Wang, X. (1996) Cell 86, 147–157
54. Zou, H., Henzel, W. J. Liu, X., Lutschg, A., and Wang, X. (1997) Cell 90, 405–413
55. Langer, R. D., Criqui, M. H., and Reed, D. M. (1992) Circulation 85, 910–915
56. Chen, C. H., Gray, M. O., and Mochly-Rosen, D. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 12784–12789
57. Corder, R., Douthwaite, J. A., Lees, D. M., Khan, N. Q., Viseu Dos Santos, A. C., Wood, E. G., and Carrier, M. J. (2001) Nature 414, 863–864