PHARMACOLOGY AND CELL METABOLISM

Vascular Actions of Nitric Oxide as Affected by Exposure to Alcohol

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Abstract — Vasodilator substances liberated from endothelial cells, mainly nitric oxide (NO), play important roles in physiologically regulating blood flow and blood pressure and preventing pathological vascular damage. Impairment of these actions promotes the genesis of cardiovascular diseases such as hypertension, cerebral and cardiac hypoperfusion, impaired vasodilatation and atherosclerosis. Low concentrations of alcohol induce increased release of NO from the endothelium due to activation and expression of NO synthase (NOS). In contrast, administration of high concentrations of alcohol or its chronic ingestion impairs endothelial functions in association with reduced NO bioavailability. The endogenous NOS inhibitor asymmetric dimethylarginine may participate in decreased synthesis of NO. Chronic alcohol intake also impairs penile erectile function possibly by interfering with endothelial, but not nitrergic nerve, function. This review article summarizes the vascular actions of NO derived from endothelial and neuronal NOS as affected by alcohol, other than wine, and acetaldehyde in healthy individuals, human materials and various experimental animals.

INTRODUCTION

There is a long history of alcohol-drinking habits, which have been evaluated to be either beneficial or harmful to health and longevity. These opposite influences are mainly dependent on the volume and term of alcohol intake as well as on other lifestyle habits and dietary factors. Knowledge about the effects of ethanol on cardiovascular functions would be quite important to establish a way to make alcohol consumption a pleasurable hobby that promotes good health and enhances longevity while minimizing untoward side effects.

Nitric oxide (NO), a labile gaseous molecule, plays a crucial role in vasodilatation, regional blood flow increase, hypotension and atherosclerosis prevention (Moncada et al., 1991). NO constitutively formed in endothelial cells via endothelial nitric oxide synthase (eNOS) and in parasympathetic perivascular nerves and brain neurons via neuronal NOS (nNOS) activates soluble guanylyl cyclase in vascular smooth muscle cells to produce cyclic GMP that is mainly involved in the vascular actions of NO. Impairment of endothelial functions participates in many cardiovascular diseases, including hypertension, stroke, coronary heart diseases and atherosclerosis (Yetik-Anacak and Catravas, 2006; Förstermann and Münzel, 2006; Liu and Huang, 2008). Interference with actions of NO derived via nNOS in the peripheral and central nervous system also causes blood pressure elevation and regional blood flow reduction (Toda and Okamura, 2003; Toda et al., 2009a, b).

Up-to-date findings about the interaction between ethanol and endothelial or nitrergic nerve functions obtained using isolated tissues and cells or under in vivo conditions in humans and experimental animals revealed that the effects of ethanol on NO availability are highly dependent on whether it is taken at low or high concentration and whether the intake is acute or chronic. This review article summarizes the information about the actions of endogenous NO on blood pressure, vascular contractility and cerebral blood flow, as affected by ethanol, other than wine, and its metabolite acetaldehyde (AcH). Although favorable influences of wines, grapes and their active ingredients on cardiovascular functions have been intensively reported, only some of the recent review articles on this topic are included in the present article.

Nitric oxide exerts its effects by increasing cyclic GMP

NO is produced when L-arginine is transformed to L-citrulline by catalysis of NO synthase (NOS) in the presence of O2 and cofactors. Ca" is required for the activation of neuronal and endothelial NOS (nNOS and eNOS) but not inducible NOS (iNOS). nNOS is constitutively expressed in the brain (Breit and Snyder, 1990), peripheral nerves and kidneys, and eNOS is constitutively expressed mainly in endothelial cells (Förstermann et al., 1991). eNOS is also activated by an alternative, Ca"-independent pathway through phosphatidylinositol-3 kinase and serine/threonine protein kinase Akt (protein kinase B) (Dimmelmol et al., 1999) (Fig. 1). iNOS is not constitutively expressed, but is induced mainly in macrophages in response to bacterial lipopolysaccharide (LPS) and cytokines. The synthesis of NO by these NOS isoforms is inhibited by L-arginine analogs, including N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA; Palmer et al., 1988), N\textsuperscript{G}-nitro-L-arginine (L-NA; Toda et al., 1990), L-NA methyl ester (L-NAME; Rees et al., 1990) and asymmetric dimethylarginine (ADMA; Vallance et al., 1992), 7-Nitroindazol (7-NI; Moore et al., 1993) and S-methyl-L-thiocitrulline (Furmire et al., 1994) are the most promising NOS inhibitors so far introduced, and aminoguanidine has been regarded to be a selective iNOS inhibitor (Griffiths et al., 1993). Nitro compounds, such as nitroglycerin and sodium nitroprusside (SNP), are capable of liberating NO, thus being called NO donors.

NO or nitrovasodilators activate soluble guanylyl cyclase and produce cyclic guanosine monophosphate (cyclic GMP) from GTP. Cyclic GMP-mediated activation of protein kinase G appears to be involved in a reduction of intracellular Ca\textsuperscript{2+} and a decrease in the sensitivity of contractile elements to Ca\textsuperscript{2+}, resulting in muscular relaxation. Methylene blue and oxyhemoglobin scavenges NO. 1H[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one (Garthwaite et al., 1995) decreases the synthesis of cyclic...
GMP by inhibiting the guanylyl cyclase activity. Cyclic GMP is degraded by phosphodiesterase type 5 to 5'-GMP.

Endothelial NO causes vasodilatation, increased blood flow, lowered blood pressure, inhibition of platelet aggregation and adhesion, inhibition of leukocyte adhesion and reduced smooth muscle proliferation, and it acts to prevent atherosclerosis. Non-adrenergic non-cholinergic inhibitory responses to autonomic nerve stimulation are mainly mediated through NO synthesized by nNOS; NO plays a crucial role as a neurotransmitter from the peripheral efferent nerves in blood vessels (Toda and Okamura, 1990, 2003) (Fig. 1) and corpus cavernosum (Ignarro et al., 1990; Toda et al., 2005). NO signaling appears to be essential for neural plasticity, that is, long-term potentiation in the hippocampus and long-term depression in the cerebellum. NO formed by \(N\)-methyl-D-aspartate (NMDA) receptor activation diffuses to adjacent nerve terminals to modulate neurotransmitter release (Kiss and Vizi, 2001) and to arterioles directly or via glial cells (Toda et al., 2009a) to induce vasodilatation (Fig. 1).

Under pathological conditions (e.g. during inflammation), high levels of NO are produced after induction of the expression of iNOS mainly in macrophages. On the one hand, it exerts beneficial effects by acting as an anti-bacterial, anti-parasitic, anti-viral or tumorcidal agent; on the other hand, high levels of NO, if uncontrolled, elicits detrimental effects that are produced because NO reacts with concomitantly produced superoxide anions, thereby generating highly toxic compounds such as peroxynitrite.

**Wine and other alcoholic beverages are useful antioxidants**

Modest alcohol consumption, but neither zero nor more than modest intake, beneficially reduces total mortality and cardiovascular risk. Recent studies on experimental animals and humans indicate the superiority of wine over other alcoholic beverages for human health. Epidemiologic studies revealed that moderate wine drinkers have a lower cardiovascular risk and mortality than beer or liquor drinkers (Renaud et al.,...
Table 1. Effects of ethanol on NO production and endothelial function in isolated human materials and healthy subjects

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Material</th>
<th>Et-conc. or acute/chronic</th>
<th>Effects</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kuhlmann et al. 2004</td>
<td>HUVEC</td>
<td>Low (10 and 50 mM)</td>
<td>Increase in NO and cell proliferation</td>
<td>NO release by KCa channel activation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>High (100 and 150 mM)</td>
<td>Decrease in NO and cell proliferation</td>
<td>Harmful on endothelium</td>
</tr>
<tr>
<td>Liu et al. 2002</td>
<td>HUVEC</td>
<td>Low (&lt;20 mM)</td>
<td>Cell survival</td>
<td>PI3/Akt activation, eNOS activation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>High (50–100 mM)</td>
<td>Apoptosis</td>
<td>Caspase-3 activation</td>
</tr>
<tr>
<td>Singhal et al. 1999</td>
<td>Neutrophil</td>
<td>High (≥50 mM)</td>
<td>Apoptosis, iNOS expression</td>
<td>iNOS-derived NO production</td>
</tr>
<tr>
<td>Maiorano et al. 1999</td>
<td>Healthy subject</td>
<td>Chronic</td>
<td>Decrease in FMD</td>
<td>Reduced NO release from EC</td>
</tr>
<tr>
<td>Paiva et al. 2004</td>
<td>Healthy subject</td>
<td>Chronic</td>
<td>Increase in ADMA</td>
<td>Decrease in NO production</td>
</tr>
<tr>
<td>Hultberg et al. 1993</td>
<td>Healthy subject</td>
<td>Chronic</td>
<td>Increase in HC</td>
<td>Possible decrease in NO production</td>
</tr>
<tr>
<td>Cravo et al. 1996</td>
<td>Healthy subject</td>
<td>Chronic</td>
<td>Increase in IC</td>
<td>Possible decrease in NO production</td>
</tr>
<tr>
<td>Sierksma et al. 2003</td>
<td>Healthy subject</td>
<td>Acute Chronic</td>
<td>Plasma NO decrease</td>
<td>Attenuation of food-induced NO release</td>
</tr>
<tr>
<td>Di Gennaro et al. 2007</td>
<td>Healthy subject (alcoholic)</td>
<td>Alcohol withdrawal 37 months</td>
<td>Decrease in FMD Hypertension</td>
<td>Endothelial dysfunction, persisted</td>
</tr>
</tbody>
</table>

Et-conc., ethanol concentration; HUVEC, human umbilical vein endothelial cell; KCa channel, Ca\(^{2+}\)-activated K\(^+\) channel; PI3, phosphatidylinositol-3 kinase; Akt, serine/threonine protein kinase Akt; FMD, flow-dependent dilatation; EC, endothelial cell; HC, homocysteine.

1999; de Gaetano et al., 2002; Klatsky et al., 2003). Favorable effects of resveratrol, procyanidins and other ingredients in wine or grapes, together with ethanol itself, may contribute to the better efficacy (Opie and Lecour, 2007). According to Karatzi et al. (2004), red wine that contained no alcohol resulted in higher flow-mediated vasodilatation than regular red wine. As useful review articles on beneficial effects of wine have already been published (Parks and Booyse, 2002; Stoclet et al., 2004; Opie and Lecour, 2007; Toda, 2007b), this article is mainly devoted to discussing the vascular effects of alcoholic beverages other than wine in reference to the NO/cyclic GMP pathway.

Studies on human tissues and cells and healthy individuals

Studies on isolated arteries and endothelial cells. Detailed analyses of alcohol actions and its mechanism of action on isolated human arteries and cultured endothelial cells have been performed.

In cultured human umbilical vein endothelial cells (HUVEC), ethanol (10 and 50 mM) caused increases in NO levels and HUVEC proliferation, but high concentrations (100 and 150 mM) reduced NO synthesis and endothelial proliferation; the effects of low concentrations of ethanol were inhibited by the selective Ca\(^{2+}\)-activated K\(^+\) channel inhibitor iberiotoxin (Kuhlmann et al., 2004). These findings indicate a possible beneficial effect of low-dose ethanol on endothelial function, whereas higher concentrations may be considered as harmful. Exposure of HUVEC to ethanol resulted in rapid induction of Akt phosphorylation that was prevented by pertussis toxin or the PI3 kinase inhibitor wortmannin; low concentrations of ethanol increased eNOS activity, which was blocked by transfection of HUVEC with dominant-negative Akt, implicating the PI3 kinase/Akt pathway in the effect (Liu et al., 2002). Furthermore, incubation of HUVEC with high concentrations of ethanol resulted in mitochondrial permeability transition and caspase-3 activation followed by apoptosis. It appears that low concentrations of ethanol activate cell survival by promoting the PI3 kinase/Akt pathway in endothelial cells, whereas the proapoptotic caspase pathway is activated by higher concentrations of ethanol (Table 1). Kay et al. (2000) provided evidence suggesting that exposure of the human placental villous tissue to ethanol (50–200 mM) decreased tissue cyclic GMP content and NO release into the effluent possibly by increased oxidative stress. Decreased NO availability may adversely affect placental blood flow regulation, which in turn accounts for the growth restriction seen in ethanol-exposed fetuses. Umbilical cord arteries obtained from ethanol-exposed mothers developed less contractile force in response to serotonin or high K\(^+\) than those of non-exposed mothers, and this difference persisted even after endothelial releases of NO and PG\(_{12}\) were inhibited; 66% of the neonates in the ethanol-exposed group presented at least one minor malformation, whereas only 16% of the non-exposed group did (Iveli et al., 2007). The authors suggested that even light drinking should be considered a risk during pregnancy. NO and prostanoids do not seem to participate in the genesis of ethanol-induced malformation.

Human neutrophils harvested from healthy subjects after an alcohol drinking binge showed enhanced apoptosis; in in vitro studies, ethanol (≥50 mM) accelerated the apoptosis of human and rat neutrophils; L-NMMA and L-NAME attenuated the ethanol-induced apoptosis; and ethanol enhanced neutrophil expression of iNOS and stimulated neutrophil NO generation (Singhal et al., 1999). The enhanced neutrophil apoptosis by ethanol appears to be mediated through the generation of NO.

In vivo studies on humans. Mechanisms underlying impairment of NO-mediated vasodilatation by chronic alcohol intake in humans have been analyzed.
Brachial artery flow-mediated vasodilatation after cuff occlusion was less in alcohol abusers than in alcohol-free subjects, whereas no difference was noted when nitroglycerin was administered, suggesting an impairment of endothelium-dependent vasodilatation in chronic alcohol intake (Maiorano et al., 1999). Studies on hypercholesterolemic women revealed that alcohol drinkers had higher plasma ADMA concentration than abstainers (Paiva et al., 2004). A high plasma concentration of this endogenous NOS inhibitor is suggested to be a marker of risk for endothelial dysfunction and cardiovascular diseases (Bögér, 2003; Vallance and Leiper, 2004). There is evidence implicating that increased ADMA concentrations in plasma impair cerebral hemodynamics through inactivation of eNOS and nNOS (Dayoub et al., 2008). Higher concentrations of plasma homocysteine were noted in alcohol drinkers compared with abstainers (Hultberg et al., 1993). In chronic alcoholics, serum pyridoxal 5'-phosphate and red blood cell folate concentrations were lower and serum homocysteine levels were higher than in non-drinkers (Cravo et al., 1996). There are a number of papers indicating that elevated plasma levels of ADMA are associated with hyperhomocysteinemia and endothelial dysfunction (reviewed by Lentz et al., 2003). Homocysteine inhibits eNOS-dependent vasodilatation and blood flow (Toda, 2007a). On the other hand, in patients with coronary artery disease, alcohol consumption (light and moderate amounts) increased brachial artery diameter responses to hyperemic flow but not the response to nitroglycerin, whereas high amounts (>51 g/day) of alcohol intake had no effect on the endothelium-dependent responses (Teragawa et al., 2002). Endothelial function appears to be improved by light to moderate alcohol intake in these patients.

In healthy, non-smoking middle-aged men, serum NOx concentrations were lower at 1 h after dinner with alcoholic beverages compared with those after dinner with water; chronic moderate alcohol consumption had no effect on serum NOx concentrations (Sierksma et al., 2003). In regular beer drinkers, there was no change in the vitamin E content of LDL or its cholesterol and protein contents during the low alcohol period (0.9% alcohol for 4 weeks), whereas analysis of LDL oxidation kinetics revealed an increase in oxidizability during the high alcohol phase (4.9% alcohol for 4 weeks) (Croft et al., 1996). Enhanced oxidative stress can participate in impairment of endothelial function. As compared with the lifetime alcohol-abstaining control subjects, the flow-mediated brachial artery dilatation was reduced in detoxified alcoholics (~37 months after withdrawal) who also showed higher blood pressure, uric acid, endothelin-1 and fasting insulin, suggesting that previous heavy alcoholism, in spite of long-term withdrawal, is associated with endothelial dysfunction and a wide cluster of hemodynamic, vascular and metabolic abnormalities (Di Gennaro et al., 2007).

Table 1 summarizes the information concerning endothelial functions in human tissues and cells and healthy subjects as affected by ethanol. It is evident that low concentrations of ethanol are beneficial to endothelial cells, whereas high concentrations impair endothelial functions and cell viability. Chronic alcohol drinkers so far reported have impaired endothelial function even after long-term withdrawal; the impairment appears to result from direct effects of ethanol on the endothelial cells and from the production of detrimental substances (ADMA and homocysteine) and enhanced oxidative stress that are expected to interfere with NO synthesis or scaveng NO.

Studies on experimental animals

Systemic blood pressure. Excess alcohol intake is suggested to cause an increase in blood pressure (Klatsky et al., 1977; Marmot et al., 1994; Ohmori et al., 2002; Okamura et al., 2004). There is a relationship between average weekly alcohol consumption, blood pressure level and hypertension prevalence (Beilin and Puddey, 2006).

Male Fisher rats orally given 20% ethanol (4 g/kg) for 12 weeks showed increased systolic and diastolic blood pressure compared with controls; in the thoracic aorta isolated from ethanol-fed rats, eNOS and vascular endothelial growth factor expressions were down-regulated, leading to depletion of aortic NO levels; the aortic NADPH oxidase activity was enhanced with a concomitant increase in membrane lipid peroxidation; and the vasorelaxation in response to ACh was diminished (Husain, 2007). Chronic ethanol ingestion is implicated to induce aortic endothelial oxidative injury and down-regulation of the NO generating system, leading to impaired vasorelaxation and hypertension. On the other hand, Kleinhenz et al. (2008) noted that male Sprague–Dawley rats that chronically ingested ethanol (36% of their daily caloric intake) for 6 weeks showed decreased mean blood pressure; NO production was increased, relaxation responses to ACh were enhanced and eNOS expression was increased in the aortas of these ethanol-fed rats. Further studies are required to determine if the contrasting effects of ethanol are due to differences in the length of intake (12 vs 6 weeks), strains or nursing conditions.

In conscious female rats, intragastric administration of ethanol (0.5 and 1 g/kg) elicited an increase in NO in the nucleus tractus solitarius and a reduction of blood pressure, whereas these effects were absent in ovariectomized rats; in ovariectomized rats pretreated with estrogen, the neurochemical and blood pressure effects of ethanol were restored, suggesting that the increased production of NO via rapid estrogen signaling in the nucleus tractus solitarius neurons contributes to ethanol-induced hypotension (Li and Abdel-Rahman, 2009). There is evidence indicating that increased production of NO in the brainstem reduces central sympathetic outflow to the periphery, leading to hypotension (Sandor et al., 1995).

Blood vessels. There are literature reports describing the acute effects and mechanisms of action of alcohol in various arteries from different animal species on NO-mediated vasodilatation and modulation of vasodilator responses to endogenous NO of arteries isolated from animals chronically treated with alcohol.

In isolated bovine pulmonary arteries, ethanol (0.01–1.28%) increased the content of NO released under basal conditions and enhanced bradykinin (BK)-induced release of NO from the endothelium (Greenberg et al., 1993). Exposure of bovine pulmonary artery endothelial cells to ethanol (100 mM) for 20–120 min did not change either basal or agonist-stimulated NOS activity, whereas chronic exposure for 96 h increasedBK-, ATP- and ionomycin-stimulated NOS activity (Davda et al., 1993). In cultured bovine aortic endothelial cells (BAEC) under resting conditions, ethanol (0.8–160 mM) increased eNOS activity; BAEC exposed to steady perfusion flow exhibited a flow-dependent increase in eNOS activity, which was further enhanced by ethanol (Hendrickson et al., 1999). Ethanol increased both basal and stimulated NO production that was accompanied by an increase in eNOS protein and mRNA expression levels in BAEC (Venkov et al., 1999). Porcine pulmonary artery endothelial cells exposed for 72 h to ethanol...
and 5%), relaxant responses to the adenosine analog 5′-amino-1β-D-ribofuranoside (5′-AR) in young spontaneously hypertensive rats (SHR), in which blood pressure was increased compared with those from SHR without ethanol treatment; these responses were dependent on the endothelium and attenuated by L-NMMA (Rekik et al., 2002). Ethanol-induced hypotension may be due to an enhancement of NO-dependent vasodilatation associated with chronic treatment with ethanol. According to Köhönen et al. (1999), ethanol consumption (25% ethanol by intragastric gavage) for 5 weeks in young 3-months-old rats was associated with improved relaxations of mesenteric arterial rings to isoprenaline and cromakalim, a hyperpolarizing vasodilator acting via ATP-sensitive K+ channels, as well as associated with augmented relaxations to ACh during inhibition of NOS and cyclooxygenase, suggesting that the K+ channel-related component of arterial relaxation appears to be augmented by long-term ethanol exposure. Relaxations induced by ACh, but not SNP, were increased in aortic rings isolated from rats fed diets containing ethanol for 4 weeks, which may contribute to minimize the blood pressure elevation associated with chronic alcohol consumption (Utkan et al., 2001).

Contrary to the findings indicating beneficial effects of acute and chronic treatment with ethanol so far introduced, there is evidence suggesting its action to impair endothelial functions. Endothelium-dependent vasodilatation induced by ACh and ATP in isolated perfused rat mesenteric arteries was abolished by infusion of ethanol (7.9 mg/ml) for 60 min, but papaverine-induced vasodilatation was unaffected; histological examination revealed the presence of endothelial cells in ethanol-treated and control arterial beds (Criscone et al., 1989). Similar results were also obtained in isolated rat aortic strips (Hatake et al., 1989) and rat tail and mesenteric arteries (Brizzolara et al., 1994).

In summary, many reports in the literature revealed that ethanol increases the synthesis/release of NO in isolated arteries and cultured endothelial cells. On the other hand, some investigators noted that ethanol decreases endothelium-dependent relaxation in isolated arteries. The inhibitory effects of ethanol have not been determined in cultured endothelial cells from experimental animals. Because possible damage of the endothelium and generation of reactive oxygen species (ROS) or other vasoconstrictor factors cannot be excluded in experiments performed with isolated arteries, vasodilator responses to NO under basal and stimulated conditions would be underestimated. Studies on cultured endothelial cells are expected to provide direct evidence for NO synthesis/release by ethanol; however, modulations of the endothelial function during cell culture may not be ruled out.

Cerebral blood flow. NO is one of the important factors controlling cerebral blood flow (Toda et al., 2009a). Cerebral hypoperfusion causes neuronal degeneration, cognitive failure and memory loss. Impaired NO synthesis and action in brain vasculature, if induced by chronic alcohol intake, may become a triggering factor for Alzheimer’s disease (Toda et al., 2009c).

There was evidence suggesting that acute exposure (60 min) of rat pial arterioles to 20–60 mM ethanol did not alter vasodilator responses to ADP, ACh or histamine and to NMDA, but exposure to higher concentrations of ethanol (80 and 100 mM) produced impairment of dilatation to agonists that stimulate the synthesis/release of NO from the endothelium and neurons (Mayhan and Didion, 1995). In isolated, cannulated rat intracerebral arteries, ethanol (3–100 mM) caused a dose-dependent constriction; however, the mechanism of ethanol action was not analyzed (Gordon et al., 1995). Dilatation of rat basilar arteries or pial arterioles to ACh, BK and ADP was less in rats fed alcohol (36% calories for 8 days) compared to non-alcohol-fed rats; superoxide dismutase reversed the depressed endothelial NO-mediated vasodilatation in alcohol-fed rats, suggesting that impaired NO-dependent cerebral vasodilatation during chronic alcohol consumption may be related to enhanced release of ROS (Sun and Mayhan, 2001a, b). Impaired vasodilatation in response to ACh and ADP in alcohol-fed rats was also improved by the NAD(P)H oxidase inhibitor apocynin (Sun et al., 2006). These authors (Sun et al., 2002, 2006a) obtained further evidence indicating that chronic alcohol consumption impaired nNOS-dependent pial arteriolar dilatation in response to NMDA and kinin. Compared to control (non-alcohol-fed) rats, topical application of ACh and ADP produced dilatation of pial arterioles to a less extent in alcohol (36% calories)-fed male and female rats (blood alcohol concentrations of ~20 mM), where-
as the magnitude of vasodilatation in response to NMDA and kainate was less in alcohol-fed male, but not female, rats; NO donors produced similar vasodilatation in non-alcohol-fed and alcohol-fed male and female rats; application of tetrahydrobipterin improved impaired eNOS-dependent vasodilatation in alcohol-fed male and female rats (Sun and Mayhan, 2005). There appears to be a sex difference in pial arteriolar responsiveness to nNOS-dependent agonists in rats chronically exposed to alcohol. Impaired eNOS-dependent vasodilatation by long-term alcohol consumption may be related to a deficiency of tetrahydrobipterin utilization.

In summary, chronic intake of large amounts of alcohol appears to interfere with the bioavailability of NO derived from the endothelium, autonomic nitrergic nerves and brain neurons, resulting in cerebral hyperperfusion. Increased formation of ROS and ADMA may be involved in impairment of cerebral hemodynamics and brain cell viability. Cerebral neurovascular dysfunction in relation to bioavailability of NO formed by eNOS and nNOS isoforms contributes to cognitive decline and neurodegeneration in Alzheimer’s disease (reviewed by Toda et al., 2009c).

Prenatal alcohol exposure. Intravenous administration of ethanol to pregnant monkeys caused a marked collapse of umbilical vasculature that resulted in severe hypoxia and acidosis in the fetus (Mukherjee and Hodgen, 1982). Whether impaired availability of NO was involved in the genesis of ethanol-induced vasoconstriction was not determined; however, the analysis of mechanisms underlying impairment of umbilical circulation associated with alcohol intake in reference to NO synthesis and action is quite intriguing and provides useful information about alcohol toxicity in the fetus.

In mesenteric arteries isolated from female pregnant mice, the NO-mediated relaxation induced by methacholine was greater compared with those from non-pregnant mice; ethanol treatment (liquid diet of 25% ethanol-derived calories for 13 days from gestational days 6–18) attenuated the response only in the pregnant mice. Phenylephrine-induced vasoconstriction was blunted in pregnancy, possibly due to enhanced NO modulation, which was impaired by ethanol exposure (Cook et al., 2001). Female rats were fed an ethanol (36% calories)-containing liquid diet from gestation day 2 until labor; the pups continued to receive a standard chow through adulthood, and then aortic ring segments were excised; the endothelium-dependent relaxation to carbamylicholine was attenuated by prenatal ethanol exposure, but relaxations in response to SNP were similar between the control and prenatal-ethanol-exposed groups (Turcotte et al., 2002). Prenatal ethanol exposure may contribute to impairment of endothelial functions. As previously mentioned, cerebral hyperperfusion induced by chronic ethanol exposure appears to be derived also from endothelial dysfunction (Sun and Mayhan, 2005). Prenatal exposure of rats to ethanol decreased the areal density of NOS-positive neurons in the superior colliculus and the dorsolateral column of the periaqueductal gray when determined at postnatal day 35, compared with control dams without ethanol exposure (Phillips et al., 2000). Impairment of endothelium-dependent vasodilatation and decrease in nitrergic neurons in the brain may participate in the adverse effects associated with prenatal alcohol exposure. Prenatal alcohol exposure (1.5 g/kg for 30 days) during the second trimester in pregnant ewes attenuated fetal cerebral blood flow to hypoxia in the third trimester; cerebral oxygen delivery was decreased during hypoxia to a greater degree in the alcohol-exposed fetuses than in the saline-infused ones (Mayock et al., 2007). Synthesis and actions of vasodilator factors, such as endothelial NO (Coumans et al., 2003) and adenosine (Blood et al., 2003), liberated in response to hypoxia may be impaired by binge alcohol exposure.

In response to LPS, splenic norepinephrine turnover was increased in young rats exposed to alcohol in utero (pregnant dams fed an alcohol diet; 35% calories) but not in control rats, and L-NAME reversed this difference; splenic iNOS protein and plasma NO metabolite levels were increased in response to LPS to a greater extent in rats exposed to alcohol in utero (Gottesfeld et al., 1998). An augmented NO formation in rats subjected to intruterine exposure to alcohol likely accounts for the blunted sympathetic response to endotoxin. In response to LPS, young rats exposed to alcohol in utero responded with a greater increase in ileal iNOS activity and immunoreactivity (Weisbrodt et al., 1999).

Other tissues. Chronic and excessive alcohol consumption is associated with liver diseases. Hepatic NOS activity was reduced in alcohol-treated (3% for 12 weeks) rats with evidence of liver injury, but there was no difference in the hepatic expression of eNOS between ethanol-fed and control rats (Wang and Abdel-Rahman, 2005). Furthermore, the inhibitory protein caveolin-1 in the liver was increased in ethanol-treated rats, whereas the stimulatory protein calmodulin remained unchanged. The authors suggested that chronic ethanol intake attenuates hepatic eNOS activity by increasing the expression of the inhibitory protein caveolin-1 and enhancing its binding with eNOS. Greenberg et al. (1996) provided evidence suggesting that intraperitoneal ethanol inhibited the acute phase cardiovascular depression and the induction of NO characteristic of endotoxemia induced by LPS and that ethanol-induced suppression of LPS-mediated cardiovascular depression seems to be mediated through suppressing the release of intracellular platelet activating factor.

In isolated corpus cavernosum strips, NO-mediated endothelium-dependent relaxations in response to ACh were abolished but those induced by electrical field stimulation were unaffected in mice treated with ethanol (95% ethanol infused at 0.0035 ml/min after exposure for 7 or 14 days to ethanol vapor); endothelial damage in these ethanol-treated mice were demonstrated by electron microscopy (Aydinoglu et al., 2008). Erectile dysfunction is caused by a variety of pathogenic factors, particularly impaired formation and action of NO from nitrergic nerves and endothelial cells (Toda et al., 2005). Ethanol treatment appears to be responsible for impairment of endothelial function in the corpus cavernosum; nNOS and NO actions may not be influenced.

Acetaldehyde

Most ACh is generated in the liver by alcohol dehydrogenase during ethanol metabolism. ACh may have effects locally or when delivered to other cells by the bloodstream. ACh in the blood vessel tissues are suggested to participate in the toxic cardiovascular effect and the flushing response; on the other hand, some ACh effects, such as the vascular release of NO and PGI2, are expected to take part in the protection by moderate alcohol consumption against some cardiovascular complications (Allali-Hassani et al., 1997).

Induction of NOS and ACh dehydrogenase in Purkinje cells, basket-like neurons and microvascular endothelium of
human cerebellar cortex was detected in patients with chronic alcohol intoxication (Konovko et al., 2004). NO donors such as S-nitrosoglutathione, S-nitroso-N-acetylpenicillamine and 3-morpholinosydnonimine increased the nitrite concentration while they inhibited the mitochondrial ACh dehydrogenase activity in rat hepatoma cells; addition of glutathione-ethylester blocked the S-nitrosoglutathione-mediated ACh dehydrogenase inhibition and increased nitrite concentration, suggesting that S-nitrosylation of mitochondrial ACh dehydrogenase in intact cells leads to reversible inhibition of ACh dehydrogenase activity (Moon et al., 2005). There is evidence suggesting that the vascular endothelium contributes to extrahepatic metabolism of ACh (Tampier et al., 1993).

Administration of ACh exerted relaxations in isolated rat aortic strips and portal veins (Altura et al., 1978). Mechanisms underlying the response were not determined. Coronary vasodilator effects of ACh were noted in anesthetized dogs (Bandow et al., 1977). Isolated dog mesenteric arteries responded to ACh with relaxations that were attenuated by cyclooxygenase inhibitors (Toda et al., 1983). ACh stimulated the PGI₂ release from rat aortic rings; ethanol did not release PGI₂ in control rat aortas, but did stimulate its release from rats chronically fed liquid diets containing ethanol (Altura et al., 1983). ACh also increased the NO production in human endothelial cells, so far reported. Endothelium-dependent, NO-mediated cerebral arteriolar dilatation, as well as vasodilatation mediated by nNOS-derived NO, is impaired by chronic alcohol consumption; increased ROS production and depletion of tetrahydrobiopterin may be involved in these effects. Similar results on endothelium-dependent vasodilatation are also observed in fetuses and pups subjected to prenatal alcohol exposure. Acetaldehyde-induced relaxation in isolated dog arteries appears to be mediated by prostacyclin. The penile erectile response to nNOS- and eNOS-derived NO is attenuated by acetaldehyde. There is still a lack of necessary information particularly about healthy subjects and human materials. Detailed analyses on the interactions between chronic ethanol intake and NO-mediated blood flow regulation are expected to provide us more reasonable indications for health-oriented alcohol-drinking habits.

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**REFERENCES**


