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Original Contribution

Evidence of cardiovascular protection by moderate alcohol: Role of nitric oxide

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Abstract

Epidemiological evidence indicates that moderate alcohol consumption reduces the incidence of heart disease. Endothelial nitric oxide synthase (eNOS) is a key regulator of vascular homeostasis and myocardial functions through the controlled production of nitric oxide ($^{\bullet}$NO). These studies were conducted to determine if the apparent alcohol-associated cardioprotection is mediated, in part, through modulation of the eNOS protein and activity in the cardiovascular system. Rats were fed alcohol and eNOS protein and $^{\bullet}$NO production were evaluated at the end of 8 weeks. Myocardial and vascular function was assessed ex vivo in a subset of animals. Moderate alcohol improved postischemic myocardial systolic and diastolic function and attenuated the postischemic reduction in coronary vascular resistance. Moderate alcohol also enhanced maximum vascular relaxation by 26 ± 0.2% and increased plasma $^{\bullet}$NO production concomitant with a greater than 2.5-fold increase in eNOS protein. Higher levels of alcohol impaired maximum vascular relaxation by 22 ± 0.1%. These results suggest that moderate alcohol improves postischemic myocardial functions and increases $^{\bullet}$NO production by vascular endothelium. An increase in $^{\bullet}$NO may explain, at least in part, the cardioprotective benefits of moderate alcohol consumption.

Keywords: Nitric oxide synthase; Endothelium; Cardioprotection; Heart; Alcohol; Cardiac function; Vasorelaxation

Introduction

Coronary heart disease (CHD) remains the leading cause of death in both men and women in the United States [24]. In patients with cardiovascular risk factors such as hypercholesterolemia, hypertension, or aging [48] endothelial dysfunction predisposes to the development of structural vascular changes [40] and may play a critical role in acute myocardial infarction (MI) and sudden death. Heavy alcohol consumption has long been associated with vascular as well

Abbreviations: CHD, coronary heart disease; MI, myocardial infarction; NO, nitric oxide; eNOS, endothelial nitric oxide synthase; nNOS, neuronal NOS; iNOS, inducible NOS; ACh, acetylcholine; EC, endothelial cell; HRP, horseradish peroxidase.

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as myocardial complications including hemorrhagic stroke, hypertension, cardiomyopathies, arrhythmias, and coronary heart disease [24]. Paradoxically, several epidemiological studies suggest an inverse association between long-term moderate alcohol consumption and the risk of CHD and MI [44]. Moderate alcohol consumption was also shown to reduce all causes of mortality by almost one-third [14]. Peripheral artery disease (PAD) shares many pathophysiologic features with coronary and cerebral atherosclerosis, and similar to CHD, displays an inverse association between moderate alcohol consumption and the risk of vascular complications [10].

The cardioprotection associated with moderate alcohol consumption could be attributed to the effects of low-dose alcohol on various aspects of cardiovascular functions. Moderate alcohol was shown to increase high-density lipoproteins [29], decrease platelet aggregation [38], enhance fibrinolytic activity through the upregulation of tissue plasminogen activator [7], decrease fibrinogen [1,39], and decrease ischemia–reperfusion injury [30]. The antiatherogenic effects of alcohol have been attributed, at least in part, to the antioxidant effects of ethanol and in particular the scavenging of superoxide anion [19]. However, recent data suggest that ethanol does not significantly scavenge superoxide nor increase •NO through altered reaction of •NO with superoxide [20]. Despite a growing literature about the effects of alcohol, the detailed molecular mechanisms of the cardiovascular protection remain elusive [8,33].

Nitric oxide produced in the endothelium is a key regulator of vascular homeostasis, including basal vascular tone (blood flow) and blood pressure [35,37]. Nitric oxide also inhibits smooth muscle proliferation, platelet aggregation, and monocyte adhesion, making it an overall antithrombogenic agent [26]. Nitric oxide is generated by the action of three isoforms of nitric oxide synthase (NOS). Of these, endothelial NOS is the constitutive form existing primarily in vascular endothelium. Cardiac myocytes also constitutively express eNOS, which contributes to the regulation of myocardial contractility, heart rate [25], and cardiac oxygen consumption [28]. Cardiac eNOS can be activated in both atrial and ventricular myocytes by various stimuli [15] and has been postulated to play a protective role in both congestive heart failure [23] and myocardial ischemia–reperfusion [21,43]. It was the purpose of the present studies to investigate the role of chronic consumption of moderate alcohol in the upregulation of eNOS protein and its effect on vascular and postischemic myocardial function.

In this series of studies, we demonstrate that moderate alcohol consumption enhanced postischemic myocardial systolic and diastolic function as well as attenuated the ischemia-induced increase in coronary vascular resistance. Moderate alcohol consumption also increased the expression of eNOS protein in the vasculature and •NO metabolites in the blood. The increased eNOS is consistent with the observed improvement in acetylcholine-stimulated vascular relaxation. It is postulated that an increased level of •NO associated with upregulation of eNOS protein could account for the enhanced postischemic myocardial function and vascular relaxation thereby implicating •NO in the cardiovascular protection associated with moderate alcohol consumption.

Materials and methods

Biochemicals

All chemicals, unless specified, were obtained from Sigma Chemical Co. (St. Louis, MO). Polyclonal antibody against eNOS and nNOS was obtained from Transduction Laboratories (Lexington, KY). Polyclonal antibody against eNOS $Ser^{1177/1179}$ was obtained from Cell Signaling Technology, Inc. (Beverly, MA). Polyclonal antibody against eNOS $Thr^{495/497}$ was purchased from Upstate Biotechnologies (Charlottesville, VA). Secondary antibodies were obtained from Amersham-Pharmacia Biotech (Piscataway, NJ).

Animal protocols

All animal protocols were approved by the Animal Review Board of the University of Alabama at Birmingham. All animals were anesthetized prior to surgical procedures with ketamine + Rompun (10 and 1.5 mg/100 g body weight, respectively). Two basic animal alcohol administration protocols were utilized: (1) a liquid Lieber-DeCarli diet and (2) standard chow with drinking water supplemented with ethanol. The oral route of alcohol administration was chosen to mimic the human mode of intake. Weight gain and water intake was recorded 3 times per week. Blood (500 μl) was collected from all experimental animals upon anesthesia before the surgeries and stored at −80°C in a freezer.

Liquid Lieber-DeCarli diet

In a paired feeding paradigm, male Sprague-Dawley rats (225–250 g) consumed an ethanol-containing or equilcaloric liquid diet in which ethanol comprised 0, 9, or 18% of total calories for 8 weeks. These diets were prepared from a standard Lieber-DeCarli Diet (Dyets, Inc, Bethlehem, PA) that provides ~36% of the total calories of the animal diet as ethanol. The liquid diet contains 1.0 kcal/ml, 35% of which are fat derived, 47% are derived from carbohydrate, and 18% are derived from protein and is one of the most commonly used diets for alcohol research. The standard diet was mixed with a control diet in which maltose dextrin was substituted for the ethanol in the ratio of 1:1 (18%) or 1:3 (9%).

Water supplementation with alcohol

As a control for the effects of the nonalcoholic components of the liquid diet, some rats were fed a standard laboratory chow with regular water or water supplemented with 7.5% (v/v) ethanol for 8 weeks. For these studies, a modified pair-fed feeding paradigm was designed in which the solid food was weighed and the control animal intake of