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Influence of chronic alcohol consumption on inward rectifier potassium channels in cerebral arterioles

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Abstract

Inward rectifier potassium (K_{IR}) channels appear to play an important role in the regulation of cerebral blood flow. Our goal was to examine the influence of chronic alcohol exposure on K_{IR} channels in cerebral arterioles. Sprague–Dawley rats were fed liquid diets with or without alcohol for 8–12 weeks. Using intravital microscope, we measured diameter of pial arterioles in response to an inhibitor, $BaCl_2$, and an activator, KCl, of K_{IR} channels in the absence and presence of a scavenger of reactive oxygen species, tempol, or an inhibitor of NAD(P)H oxidase, apocynin. Application of $BaCl_2$ (30 and 100 μM) produced dose-related vasoconstriction in non-alcohol-fed, but not in alcohol-fed rats. In addition, application of KCl (3, 10, and 30 mM) produced dose-related dilation in non-alcohol-fed and alcohol-fed rats, but the magnitude of vasodilatation was less in alcohol-fed rats. In contrast, nitroglycerin-induced vasodilation was similar in non-alcohol-fed and alcohol-fed rats. Superfusion of cranial window with tempol (0.1 mM) or apocynin (1 mM) did not alter baseline diameter and nitroglycerin-induced dilation of pial arterioles in non-alcohol-fed and alcohol-fed rats but significantly improved impaired KCl-induced dilation in alcohol-fed rats. Our findings suggest that chronic alcohol consumption impairs the role of K_{IR} channels in basal tone and KCl-induced dilation of cerebral arterioles. In addition, impaired KCl-induced dilation of cerebral arterioles during alcohol consumption may be related to enhanced release of oxygen-derived free radicals via NAD(P)H oxidase.

Keywords: Alcohol; Cerebral circulation; Potassium channel; Oxidative stress; NAD(P)H oxidase

Introduction

Chronic alcohol consumption reduces cerebral vasoreactivity, and thus may contribute to the pathogenesis of stroke and cognitive dysfunction observed in chronic alcoholics (Brown and Tapert, 2004; Harper and Matsumoto, 2005; Nutt, 1999). In previous studies, we reported that dilation of cerebral arterioles to agonists that stimulate either endothelial or neuronal synthesis/release of nitric oxide (NO) is profoundly impaired during chronic alcohol consumption (Mayhan, 1992; Mayhan and Didion, 1996; Sun et al., 2002). In addition to the synthesis/release of NO from the endothelium and neurons, potassium (K^+) channels are present in cerebral vessels and activation of these channels may be a major mechanism of cerebral vasodilatation in response to vasoactive and physiological stimuli (Armstead, 1999a; Bolotina et al., 1994; Faraci et al., 1994; Gebremedhin et al., 1994; Toyoda et al., 1997).

Four types of K^+ channel, including ATP-sensitive (K_{ATP}), calcium-activated (K_{Ca}), voltage-dependent (K_V), and K_{IR} channels, are present in cerebral arterioles (Horiuchi et al., 2002; Sobey et al., 1998). Recent evidence indicates that K_{Ca}, K_V, and K_{IR} channels are active under basal conditions in cerebral arterioles (Gokina et al., 1996; Horiuchi et al., 2002, 2001). In addition, K_{IR} channels have been suggested to be involved in the regional coupling of cerebral blood flow to changes in cerebral metabolism (Zaritsky et al., 2000). Studies have reported that acute exposure of cultured rat aortic smooth muscle cells to alcohol inhibits K_V and K_{Ca} channels (Dopico, 2003; Walters et al., 2000; Wu and Chao, 1995). In our previous study, we found that chronic alcohol consumption impairs dilation of cerebral arterioles in response to activation of K_{ATP} (Mayhan and Didion, 1996). No studies,

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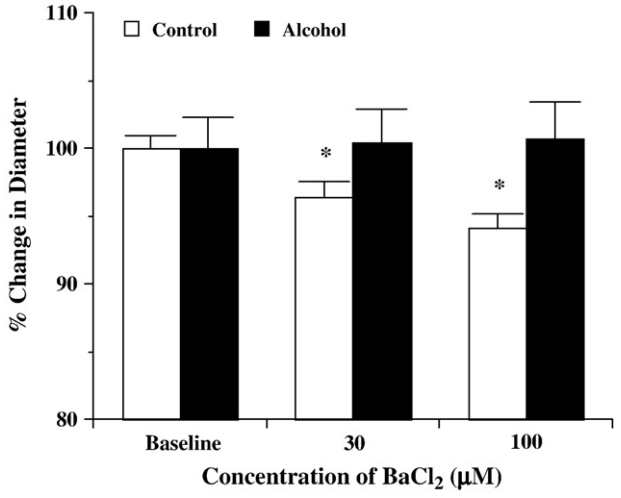

Fig. 1. Response of parietal pial arterioles to $BaCl_2$ in 2–3 months non-alcohol-fed (Control) ($n=5$) and alcohol-fed (Alcohol) ($n=5$) rats. Values are means± SEM. *$P<0.05$ vs. baseline diameter.

however, have examined the effects of chronic alcohol consumption on K_{IR} channels under basal conditions, or during stimulation of K_{IR} channels in cerebral arterioles. Thus, our first goal was to examine whether in vivo reactivity of pial arterioles to an inhibitor and an activator of K_{IR} channels is altered during chronic alcohol consumption. Our second goal was to examine a possible mechanism that may contribute to impaired reactivity of pial arterioles during alcohol consumption. Recent studies indicated that oxidative stress contributes to impairment of K^+ channel function in cerebral vasculature, coronary vasculature, and cerebral artery smooth muscle cells (Armstead, 1999b; Armstead, 2001; Bari et al., 1996; Brzezinska et al., 2000; Liu et al., 2001). Previous studies, including ours, have suggested that alcohol consumption induces oxidative stress via NAD(P)H oxidase (Kono et al., 2000; Polikandriotis et al., 2006; Sun et al., 2006a,b). Thus, we measured the effects of oxygen radical scavenger (tempol) and NAD(P)H oxidase inhibitor (apocynin) on alcohol consumption induced inhibition of K_{IR} channel function in cerebral arterioles.

Methods

Experimental diets

All procedures were in accordance with the "Principle of Laboratory Animal Care" (NIH publication No. 86-23, revised 1985) and were approved by the Institutional Animal Care and Use Committee. Male Sprague–Dawley rats were purchased from Harlan. At 2 months of age (body weight 200 to 220 g), the rats were singly housed and divided into two groups, a non-alcohol-fed group ($n=17$) and an alcohol-fed group ($n=18$). We fed rats liquid diets (Dyets, Bethlehem, PA) for 8–12 weeks. These diets have been used extensively to study the chronic effects of alcohol in rats (Lieber et al., 1989; Mayhan, 1992; McMartin et al., 1989). The nonalcohol rats were given a liquid diet that contained 1.0 kcal/ml, of which 35% were derived from fat, 47% were derived from carbohydrates, and 18% were derived from protein. Rats in the alcohol-fed groups were given a liquid diet that contained 1.0 kcal/ml, of which 35% were derived from fat, 11% were derived from carbohydrates, 18% were derived from protein, and 36% were derived from ethanol. The concentration of full dose ethanol in the diet was 6.4%. We gradually introduced full dose ethanol into the diet over a 5-day period. Ethanol was given at 1.6% during first 2 days, at 3.2% during next 3 days, and at full dose (6.4%) from the 6th day. The total daily volume of diet fed to the non-alcohol-fed rats was based on the consumption of diet by the alcohol-fed rats, and thus the daily consumption of diet was similar in the non-alcohol-fed and alcohol-fed rats.

Preparation of animals

Before the experiment, we removed liquid diet for 12 h. The rats were anesthetized with thiobutabarbital sodium (Inactin) (100 mg/kg body weight, ip), and a tracheotomy was performed. The rats were ventilated mechanically with room air and supplemental oxygen. A catheter was placed into a femoral vein for injection of supplemental anesthesia, and a femoral artery was cannulated for measurement of arterial blood pressure and to obtain a blood sample for the measurement of arterial blood gas.

To visualize the microcirculation of the cerebrum, a craniectomy was prepared over the left parietal cortex. The cranial window was suffused with artificial cerebrospinal fluid (2 ml/min) that was bubbled with 95% nitrogen and 5% carbon dioxide. Temperature of the suffusate was maintained at 37±1 °C. The cranial window was connected via a three-way valve to a pump, which was allowed for infusion of agonists and antagonists into the suffusate. This method maintained a constant temperature, pH, pCO_2, and pO_2 of the suffusate during infusion of drugs. Diameter of pial arteriolar was measured using a video image-shearing device.

Experimental protocol

Rats from each group were divided into three experimental conditions, $BaCl_2$, KCl before and during tempol, and KCl before and during apocynin. Cerebral vessels were superfused with artificial cerebral spinal fluid for 1 h before testing responses of arterioles. To examine the basal activity of K_{IR} channels and K_{IR} channel activation-induced cerebral vasodilation, we measured responses of pial arterioles to $BaCl_2$ (30 and 100 μM; an inhibitor of K_{IR} channels) and KCl (3, 10, and 30 mM; an activator of K_{IR} channels), respectively. We also examined responses to nitroglycerin (0.1 and 1 μM) in the absence and presence of tempol and apocynin. $BaCl_2$, KCl, and nitroglycerin were mixed in artificial cerebral spinal fluid and then superfused over the cranial window. Diameter of pial arterioles was measured immediately before application of antagonist and agonists and every minute for 5 min during application of antagonist and agonists. Steady-state responses to the antagonist and agonists were reached within 2–3 min after starting application and the diameter of pial arterioles returned to baseline within 5 min after application of antagonist and agonists was stopped. To examine the potential mechanism underlying impaired K_{IR} channel activation-mediated dilation during alcohol consumption, we suffused the cranial window preparation with cerebrospinal fluid containing tempol (0.1 mM) or apocynin (1 mM) after the initial measurements. One hour after starting the suffusion of tempol or apocynin, and continuing for the duration of the experiment, we again examined responses of pial arterioles to KCl and nitroglycerin.

Statistical analysis

For comparison of the various treatments, results were compared using a two-way repeated measure ANOVA with Tukey's post hoc test. Student t tests were used to compare responses to KCl and nitroglycerin before and following application of tempol or apocynin. Values are means± SEM. A p value of 0.05 or less was considered to be significant.

Results

Control conditions

Body weight (non-alcohol-fed: 426±12 g; alcohol-fed: 417±7g) and mean arterial pressure (non-alcohol-fed: 85±7 mmHg; alcohol-fed: 85±6 mmHg) were similar in both groups of rats.