Published in final edited form as:

Microvasc Res. 2008 May ; 75(3): 367–372.

Influence of Chronic Alcohol Consumption on Inward Rectifier Potassium Channels in Cerebral Arterioles

Hong Sun, Ph.D., Honggang Zhao, Ph.D., Glenda M. Sharpe, Denise M. Arrick, and William G. Mayhan, Ph.D.

Department of Cellular and Integrative Physiology, University of Nebraska Medical Center, Omaha, NE 68198-5850

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 nonalcohol-fed, but not in alcohol-fed rats. In addition, application of KCl (3, 10, and 30 mM) produced dose-related dilation in nonalcohol-fed and alcohol-fed rats, but the magnitude of vasodilatation was less in alcohol-fed rats. In contrast, nitroglycerin-induced vasodilation was similar in nonalcohol-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 nonalcohol-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; Caicoya et al., 1999; Harper and Matsumoto, 2005; Hart et al., 1999; Leppala et al., 1999; 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

Corresponding author: Dr. Hong Sun, Department of Cellular and Integrative Physiology, 985850 Nebraska Medical Center, University of Nebraska Medical Center, Omaha, NE 68198-5850, Tel: (402)-559-5040, Fax: (402)-559-4438, E-mail: hsu1@unmc.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

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; Gebramedhin 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; Horiuchi et al., 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; Walter 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, 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., 2005; Sun et al., 2006a; Sun et al., 2006b). 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 nonalcohol-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 two days, at 3.2% during next three days, and at full dose (6.4%) from the 6th day. The total daily volume of diet fed to the nonalcohol-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 nonalcohol-fed and alcohol-fed rats.

Preparation of animals

Before the experiment, we removed liquid diet for 12 hours. 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 $37\pm1^{\circ}\text{C}$. 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 one hour 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 minutes during application of antagonist and agonists. Steady-state responses to the antagonist and agonists were reached within 2–3 minutes after starting application and the diameter of pial arterioles returned to baseline within 5 minutes 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 $\pm$ SEM. A *p* value of 0.05 or less was considered to be significant.

RESULTS

Control conditions

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

Effects of alcohol consumption on basal activation of K_{IR} channels

Baseline diameter of pial arterioles was $39 \pm 1 \mu\text{m}$ in non-alcohol-fed and $38 \pm 2 \mu\text{m}$ in alcohol-fed rats ($P > 0.05$). BaCl_2 (30 and 100 μM) produced a significant vasoconstriction in nonalcohol-fed ($n=5$), but not in alcohol-fed rats ($n=5$) (Figure 1).

Effect of alcohol consumption on KCl-induced activation of K_{IR} channels

Baseline diameter of pial arterioles was $38 \pm 1 \mu\text{m}$ in nonalcohol-fed and $39 \pm 2 \mu\text{m}$ in alcohol-fed rats ($P > 0.05$). Topical application of KCl (3, 10, and 30 mM) produced a dose-related dilation of pial arterioles in nonalcohol-fed ($n=12$) and alcohol-fed rats ($n=13$). However, the magnitude of vasodilatation to KCl was significantly less in the alcohol-fed rats (Figures 2 and 3). In addition, no difference was observed in nitroglycerin-induced dilation of pial arterioles between nonalcohol-fed and alcohol-fed rats (Figure 4).

Responses following acute application of tempol and apocynin

A 1-hour topical application of tempol or apocynin did not alter baseline diameter of pial arterioles in nonalcohol-fed and alcohol-fed rats. In addition, tempol or apocynin did not alter dilation of pial arterioles in response to KCl (Figures 2 and 3) in nonalcohol-fed rats, and nitroglycerin (Figure 4) in both nonalcohol-fed and alcohol-fed rats. In contrast, topical application of tempol or apocynin significantly improved dilation of pial arterioles to KCl in alcohol-fed rats (Figures 2 and 3). However, tempol did not improve the response of pial arterioles to BaCl_2 in alcohol-fed rats (data not shown).

DISCUSSION

There are three new findings from this study. First, chronic alcohol consumption inhibits the influence of K_{IR} channels on basal diameter of parietal pial arterioles. Second, alcohol consumption impairs dilation of parietal pial arterioles in response to KCl. Third, topical application of tempol and apocynin can significantly improve alcohol consumption-induced impairment of KCl-induced dilation in parietal pial arterioles. Since activation of K_{IR} channels may contribute to the regulation of cerebral blood flow, we suggest that our findings may have implications for the control of cerebral blood flow and the pathogenesis of cerebrovascular abnormalities observed in binge drinkers and chronic alcoholics.

Many studies have shown that K^+ channels are involved in cerebral vasodilatation in response to NO, endothelium-dependent hyperpolarizing factor (EDHF), cAMP, reactive oxygen species, physiological stimuli (hypoxia and hypercapnia), and K^+ ion (Kitazono et al., 1995). Thus, K^+ channels play a major role as a mediator of cerebral vasodilatation. An increase in extracellular K^+ ion is a potent signal for the coupling of regional blood flow to the metabolic needs in the brain and many other tissue, such as heart and skeletal muscle (Haddy et al., 2006; Knot et al., 1996; Zaritsky et al., 2000). In addition, it has been reported that extracellular K^+ ion can increase to $>10 \text{ mM}$ during cerebral hypoxia and ischemia (Somjen, 1979). A modest increase in extracellular K^+ ion produces vasodilation in large and small cerebral arteries. Previously, we found that KCl (1–3 mM)-induced dilation of the basilar artery was significantly inhibited in chronic alcohol-fed rats (Sun et al., 2004). In the present study, KCl (3–30 mM)-induced dilation was similarly significantly attenuated in pial arterioles of alcohol-fed rats. KCl-induced cerebral vasodilatation is generally thought to relate to activation of vascular smooth muscle K_{IR} channels (Knot et al., 1996; Zaritsky et al., 2000). However, previous studies have reported that NO, smooth muscle K_{ATP} channel, and smooth muscle Na^+/K^+ ATPase may be partially involved in KCl-induced cerebral vasodilatation (Dreier et al., 1995; Horiuchi et al., 2002; Knot et al., 1996). Although we did not determine the contribution of NO, smooth muscle K_{ATP} channel, and smooth muscle Na^+/K^+ ATPase in KCl-induced vasodilatation during chronic alcohol consumption, our previous studies found that

alcohol consumption impairs NO-dependent and K_{ATP} channel activation-mediated dilation of cerebral arterioles (Mayhan, 1992; Mayhan and Didion, 1996; Sun et al., 2002). In addition, no studies have examined the influence of chronic alcohol consumption on cerebral vascular smooth muscle Na^+-K^+ ATPase. Thus, reduced NO availability, smooth muscle K_{ATP} channel activity, and/or smooth muscle Na^+-K^+ ATPase activity during chronic alcohol consumption cannot be excluded from impaired KCl-induced dilation of cerebral arterioles.

Various pathological processes decrease the functionality of vascular K_{IR} channels, such as hypertension, diabetes, and ischemia-reperfusion (Goto et al., 2004; Marrelli et al., 1998; Mayhan et al., 2004). Several mechanisms could account for impaired K_{IR} channel-mediated dilation of pial arterioles during chronic alcohol consumption. First, chronic alcohol consumption may alter the function of K_{IR} channels via activation of other cellular enzyme pathways, such as protein kinase C (PKC) and angiotensin II. Although the mechanisms leading the process are not understood, chronic alcohol consumption has been demonstrated to up-regulate PKC (Stubbs and Slater, 1999). In addition, alcohol consumption robustly activates the renin-angiotensin system, and increases plasma concentration of angiotensin II (Fitts and Hoon, 1993; Linkola and Ylikahri, 1979; Puddey et al., 1987; Wright et al., 1986). Recent studies found that angiotensin II inhibit the activity of K_{IR} channels through PKC in rabbit coronary arterial smooth muscle cells (Park et al., 2005; Park et al., 2006). In addition, Chrissobolis et al found that PKC inhibits K^+ -induced dilation in rat basilar artery (Chrissobolis and Sobey, 2002). Second, alcohol consumption may alter the function of K_{IR} channels via an increase in oxidative stress. Recent studies reported that tempol restored K_{Ca} function of arterial smooth muscle cells in hypertensive rats (Xu et al., 2006) and apocynin improved K_V function of small coronary arteries during oxidative stress (Bubolz et al., 2005). In addition, oxidative stress has been indicated to contribute to post-ischemic impairment of smooth muscle $Kir2.x$ current density and function (Petrault et al., 2004). Previous studies, including ours (Sun and Mayhan, 2001), suggested that alcohol consumption induces oxidative stress (Agar et al., 1999; Montoliu et al., 1994; Omodeo-Sale et al., 1997; Sun et al., 2001; Yin et al., 2001). Increased production of oxygen free radicals was found in brain, liver, and plasma of alcohol-fed rodents. Recently, we found that NAD(P)H oxidase may play an important role in alcohol consumption-induced oxidative stress in cerebral artery and cerebral cortex tissue (Sun et al., 2006a; Sun et al., 2006b). In the present study, we found that topical application with tempol or apocynin significantly improved dilation of pial arterioles in response to KCl. Thus, impaired KCl-induced dilation of cerebral arterioles during alcohol consumption may be related to increased production/release of oxygen radicals via NAD(P)H oxidase.

K_{IR} channels have been suggested to be active under basal conditions in cerebral arterioles (Horiuchi et al., 2002; Horiuchi et al., 2001). We found that topical application of $BaCl_2$ produced dose-related constriction of pial arterioles under basal conditions in nonalcohol-fed rats. However, $BaCl_2$ did not alter baseline diameter of pial arterioles in alcohol-fed rats. This finding suggests that K_{IR} channels may not be active in pial arterioles under basal condition during alcohol consumption. In general, the basal tone of pial arterioles is influenced by many regulatory systems, including endothelium-derived relaxing factors, endothelium-derived contracting factors, and K^+ channels. In our previous studies and in the present study, we did not find the difference in baseline diameter of pial arterioles between nonalcohol-fed and alcohol-fed rats (Mayhan, 1992; Mayhan and Didion, 1996; Sun et al., 2002). The explanation for this finding remains unclear but may be due to compensatory actions of other regulatory networks. In addition, topical application with tempol failed to improve the response of cerebral arterioles to $BaCl_2$ and both tempol and apocynin did not influence the baseline diameter of pial arterioles in alcohol-fed rats. Thus, it is possible that different K_{IR} channel subtype(s) may be involved in regulation of basal tone in cerebral arterioles, and it seems that inhibition of basal activation of K_{IR} channels during alcohol consumption is not related to enhanced oxidative stress and/or NAD(P)H oxidase.

In summary, alterations in reactivity of pial arterioles observed during chronic alcohol consumption may be important for the regulation of local cerebral blood flow and altered responses of pial arterioles during chronic alcohol consumption may produce detrimental effects on the brain. Studies have shown that alcohol consumption is a risk factor for the pathogenesis of stroke and cognitive dysfunction. In the present study, we found that chronic alcohol consumption inhibits basal activation of K_{IR} channels and KCl-induced dilation in cerebral arterioles. We speculate that impaired responses of cerebral vessels during alcohol consumption to activation of an important vasodilator pathway, i.e., K_{IR} channels, may have important implications for the pathogenesis of cerebrovascular abnormalities observed in chronic alcoholics.

Acknowledgements

This study was supported by National Institutes of Health Grants DA 14258, HL79587, AA 11288, a Scientist Development Grant from the American Heart Association (0635052N), and funds from the University of Nebraska Medical Center.

References

- Agar E, Bosnak M, Amanvermez R, Demir S, Ayyildiz M, Celik C. The effect of ethanol on lipid peroxidation and glutathione level in the brain stem of rat. *Neuroreport* 1999;10:1799–1801. [PubMed: 10501578]
- Armstead WM. Hypotension dilates pial arteries by K_{ATP} and K_{Ca} channel activation. *Brain Research* 1999a;816:158–164. [PubMed: 9878717]
- Armstead WM. Superoxide generation links protein kinase C activation to impaired ATP-sensitive K^+ channel function after brain injury. *Stroke* 1999b;30:153–159. [PubMed: 9880404]
- Armstead WM. Vasopressin-induced protein kinase C-dependent superoxide generation contributes to ATP-sensitive potassium channel but not calcium-sensitive potassium channel function impairment after brain injury. *Stroke* 2001;32:1408–1414. [PubMed: 11387506]
- Bari F, Louis TM, Meng W, Busija DW. Global ischemia impairs ATP-sensitive K^+ channel function in cerebral arterioles in piglets. *Stroke* 1996;27:1874–1881. [PubMed: 8841347]
- Bolotina VM, Najibi S, Palacino JJ, Pagano PJ, Cohen RA. Nitric oxide directly activates calcium-dependent potassium channels in vascular smooth muscle. *Nature* 1994;368:850–853. [PubMed: 7512692]
- Brown SA, Tapert SF. Adolescence and the trajectory of alcohol use: basic to clinical studies. *Ann N Y Acad Sci* 2004;1021:234–44. [PubMed: 15251893]
- Brzezinska AK, Gebremedhin D, Chilian WM, Kalyanaraman B, Elliott SJ. Peroxynitrite reversibly inhibits Ca^{2+} -activated K^+ channels in rat cerebral artery smooth muscle cells. *Am J Physiol Heart Circ Physiol* 2000;278:H1883–90. [PubMed: 10843885]
- Bubolz AH, Li H, Wu Q, Liu Y. Enhanced oxidative stress impairs cAMP-mediated dilation by reducing K_v channel function in small coronary arteries of diabetic rats. *Am J Physiol Heart Circ Physiol* 2005;289:H1873–80. [PubMed: 15937095]
- Chrissobolis S, Sobey CG. Inhibitory effects of protein kinase C on inwardly rectifying K^+ - and ATP-sensitive K^+ channel-mediated responses of the basilar artery. *Stroke* 2002;33:1692–7. [PubMed: 12053013]
- Dopico AM. Ethanol sensitivity of $BK(Ca)$ channels from arterial smooth muscle does not require the presence of the beta 1-subunit. *Am J Physiol Cell Physiol* 2003;284:C1468–80. [PubMed: 12570985]
- Faraci FM, Breese KR, Heistad DD. Cerebral vasodilation during hypercapnia: role of glibenclamide sensitive potassium channels and nitric oxide. *Stroke* 1994;25:1679–1683. [PubMed: 8042220]
- Fitts DA, Hoon RG. Ethanol-induced changes in plasma proteins, angiotensin II, and salt appetite in rats. *Behav Neurosci* 1993;107:339–45. [PubMed: 8484898]
- Gebremedhin D, Bonnet P, Greene AS, England SK, Rusch NJ, Lombard JH, Harder DR. Hypoxia increases the activity of Ca^{2+} -sensitive K^+ channels in cat cerebral arterial muscle cell membranes. *Pflugers Archives* 1994;428:621–630. [PubMed: 7838685]

- Gokina NI, Wellman TD, Bevan RD, Walters CL, Penar PL, Bevan JA. Role of Ca(2+)-activated K⁺ channels in the regulation of membrane potential and tone of smooth muscle in human pial arteries. *Circ Res* 1996;79:881–6. [PubMed: 8831514]
- Goto K, Rummery NM, Grayson TH, Hill CE. Attenuation of conducted vasodilatation in rat mesenteric arteries during hypertension: role of inwardly rectifying potassium channels. *J Physiol* 2004;561:215–31. [PubMed: 15550469]
- Haddy FJ, Vanhoutte PM, Feletou M. Role of potassium in regulating blood flow and blood pressure. *Am J Physiol Regul Integr Comp Physiol* 2006;290:R546–52. [PubMed: 16467502]
- Harper C, Matsumoto I. Ethanol and brain damage. *Curr Opin Pharmacol* 2005;5:73–8. [PubMed: 15661629]
- Horiuchi T, Dietrich HH, Hongo K, Goto T, Dacey RG. Role of endothelial nitric oxide and smooth muscle potassium channels in cerebral arteriolar dilation in response to acidosis. *Stroke* 2002;33:844–849. [PubMed: 11872913]
- Horiuchi T, Dietrich HH, Tsugane S, Dacey RG. Role of potassium channels in regulation of brain arteriolar tone. Comparison of cerebrum versus brain stem. *Stroke* 2001;32:218–224. [PubMed: 11136940]
- Kitazono T, Faraci FM, Taguchi H, Heistad DD. Role of potassium channels in cerebral blood vessels. *Stroke* 1995;26:1713–1723. [PubMed: 7660420]
- Knot HJ, Zimmermann PA, Nelson MT. Extracellular K⁽⁺⁾-induced hyperpolarizations and dilatations of rat coronary and cerebral arteries involve inward rectifier K⁽⁺⁾ channels. *J Physiol* 1996;492 (Pt 2):419–30. [PubMed: 9019539]
- Kono H, Rusyn I, Yin M, Gabele E, Yamashina S, Dikalova A, Kadiiska MB, Connor HD, Mason RP, Segal BH, Bradford BU, Holland SM, Thurman RG. NADPH oxidase-derived free radicals are key oxidants in alcohol-induced liver disease. *J Clin Invest* 2000;106:867–72. [PubMed: 11018074]
- Lieber CS, DeCarli LM, Sorrell MF. Experimental methods of ethanol administration. *Hepatology* 1989;10:501–510. [PubMed: 2673971]
- Linkola J, Fyhrquist F, Ylikahri R. Renin, aldosterone and cortisol during ethanol intoxication and hangover. *Acta Physiol Scand* 1979;106:75–82. [PubMed: 463581]
- Liu Y, Terata K, Rusch NJ, Gutterman DD. High glucose impairs voltage-gated K⁽⁺⁾ channel current in rat small coronary arteries. *Circ Res* 2001;89:146–52. [PubMed: 11463721]
- Marrelli SP, Johnson TD, Khorovets A, Childres WF, Bryan RM Jr. Altered function of inward rectifier potassium channels in cerebrovascular smooth muscle after ischemia/reperfusion. *Stroke* 1998;29:1469–74. [PubMed: 9660405]
- Mayhan WG. Responses of cerebral arterioles during chronic alcohol exposure. *American Journal of Physiology* 1992;262:H787–H791. [PubMed: 1558188]
- Mayhan WG, Didion SP. Effect of chronic alcohol consumption on responses of cerebral arterioles. *Alcoholism: Clinical and Experimental Research* 1996;20:538–542.
- Mayhan WG, Mayhan JF, Sun H, Patel KP. In vivo properties of potassium channels in cerebral blood vessels during diabetes mellitus. *Microcirculation* 2004;11:605–13. [PubMed: 15513870]
- McMartin KE, Collins TD, Eisenga BH, Fortney T, Bates WR, Bairnsfather L. Effects of chronic ethanol and diet treatment on urinary folate excretion and development of folate deficiency in the rat. *Journal of Nutrition* 1989;119:1490–1497. [PubMed: 2585139]
- Montoliu C, Valles S, Renau-Piquieras J, Guerri C. Ethanol-induced oxygen radical formation and lipid peroxidation in rat brain: effect of chronic alcohol consumption. *Journal of Neurochemistry* 1994;63:1855–1862. [PubMed: 7931342]
- Nutt D. Alcohol and the brain. *British journal of psychiatry* 1999;175:114–119. [PubMed: 10627792]
- Omodeo-Sale F, Gramigna D, Campaniello R. Lipid peroxidation and antioxidant systems in rat brain: effect of chronic alcohol consumption. *Neurochemical Research* 1997;22:577–582. [PubMed: 9131636]
- Park WS, Han J, Kim N, Youm JB, Joo H, Kim HK, Ko JH, Earm YE. Endothelin-1 inhibits inward rectifier K⁺ channels in rabbit coronary arterial smooth muscle cells through protein kinase C. *J Cardiovasc Pharmacol* 2005;46:681–9. [PubMed: 16220076]

- Park WS, Kim N, Youm JB, Warda M, Ko JH, Kim SJ, Earm YE, Han J. Angiotensin II inhibits inward rectifier K⁺ channels in rabbit coronary arterial smooth muscle cells through protein kinase Calpha. *Biochem Biophys Res Commun* 2006;341:728–35. [PubMed: 16442501]
- Petrault O, Bastide M, Cotel N, Gele P, Gautier S, Laprais M, Vamecq J, Duriez P, Bordet R. The neuroprotective effect of the antioxidant flavonoid derivate di-tert-butylhydroxyphenyl is parallel to the preventive effect on post-ischemic Kir2.x impairment but not to post-ischemic endothelial dysfunction. *Naunyn Schmiedebergs Arch Pharmacol* 2004;370:395–403. [PubMed: 15502971]
- Polikandriotis JA, Rupnow HL, Elms SC, Clemmus RE, Campbell DJ, Sutliff RL, Brown LA, Guidot DM, Hart CM. Chronic Ethanol Ingestion Increases Superoxide Production and NADPH Oxidase Expression in the Lung. *Am J Respir Cell Mol Biol*. 2005
- Puddey IB, Beilin LJ, Vandongen R. Regular alcohol use raises blood pressure in treated hypertensive subjects. A randomised controlled trial. *Lancet* 1987;1:647–51. [PubMed: 2882082]
- Sobey CG, Heistad DD, Faraci FM. Potassium channels mediate dilatation of cerebral arterioles in response to arachidonate. *American Journal of Physiology* 1998;275:H1606–H1612. [PubMed: 9815067]
- Somjen GG. Extracellular potassium in the mammalian central nervous system. *Annu Rev Physiol* 1979;41:159–77. [PubMed: 373587]
- Stubbs CD, Slater SJ. Ethanol and protein kinase C. *Alcoholism: Clinical and Experimental Research* 1999;23:1552–1560.
- Sun AY, Ingelman-Sundberg M, Neve E, Matsumoto H, Nishitani Y, Minowa Y, Fukui Y, Bailey SM, Patel VB, Cunningham CC, Zima T, Fialova L, Mikulikova L, Popov P, Malbohan I, Janebova M, Nespor K, Sun GY. Ethanol and oxidative stress. *Alcoholism: Clinical and Experimental Research* 2001;25:237S–243S.
- Sun H, Fang Q, Mayhan WG. Inward rectifier potassium channels in the basilar artery during chronic alcohol consumption. *Alcohol Clin Exp Res* 2004;28:1557–61. [PubMed: 15597089]
- Sun H, Mayhan WG. Temporal effect of alcohol consumption on reactivity of pial arterioles: role of oxygen radicals. *American Journal of Physiology* 2001;280:H992–H1001. [PubMed: 11179040]
- Sun H, Molacek E, Zheng H, Fang Q, Patel KP, Mayhan WG. Alcohol-induced impairment of neuronal nitric oxide synthase (nNOS)-dependent dilation of cerebral arterioles: role of NAD(P)H oxidase. *J Mol Cell Cardiol* 2006a;40:321–8. [PubMed: 16403412]
- Sun H, Patel KP, Mayhan WG. Impairment of neuronal nitric oxide synthase-dependent dilatation of cerebral arterioles during chronic alcohol consumption. *Alcoholism: Clinical and Experimental Research* 2002;26:663–670.
- Sun H, Zheng H, Molacek E, Fang Q, Patel KP, Mayhan WG. Role of NAD(P)H Oxidase in Alcohol-Induced Impairment of Endothelial Nitric Oxide Synthase-Dependent Dilation of Cerebral Arterioles. *Stroke* 2006b;37:495–500. [PubMed: 16373635]
- Toyoda K, Fujii K, Ibayashi S, Kitazono T, Nagao T, Fujishima M. Role of ATP-sensitive potassium channels in brain stem circulation during hypotension. *American Journal of Physiology* 1997;273:H1342–H1346. [PubMed: 9321824]
- Walters FS, Covarrubias M, Ellingson JS. Potent inhibition of the aortic smooth muscle maxi-K channel by clinical doses of ethanol. *American Journal of Physiology* 2000;279:C1107–C1115. [PubMed: 11003591]
- Wright JW, Morseth SL, Abhold RH, Harding JW. Elevations in plasma angiotensin II with prolonged ethanol treatment in rats. *Pharmacol Biochem Behav* 1986;24:813–8. [PubMed: 3012594]
- Wu SN, Chao CT. Inhibitory effect of ethanol on voltage-dependent potassium currents in single aortic smooth muscle cells. *Gaoxiong Yi Xue Ke Xue Za Zhi* 1995;11:514–20. [PubMed: 7474035]
- Xu H, Jackson WF, Fink GD, Galligan JJ. Activation of potassium channels by tempol in arterial smooth muscle cells from normotensive and deoxycorticosterone acetate-salt hypertensive rats. *Hypertension* 2006;48:1080–7. [PubMed: 17060504]
- Yin M, Gabele E, Wheeler MD, Connor H, Bradford BU, Dikalova A, Rusyn I, Mason R, Thurman Rg. Alcohol-induced free radicals in mice: direct toxicants or signaling molecules. *Hepatology* 2001;34:935–942. [PubMed: 11679964]

Zaritsky JJ, Eckman DM, Wellman GC, Nelson MT, Schwarz TL. Targeted disruption of Kir2.1 and Kir2.2 genes reveals the essential role of the inwardly rectifying K(+) current in K(+)-mediated vasodilation. *Circ Res* 2000;87:160–6. [PubMed: 10904001]

Figure 1.

Response of parietal pial arterioles to BaCl₂ in 2–3 months nonalcohol-fed (Control) (n=5) and alcohol-fed (Alcohol) (n=5) rats. Values are means ± SEM. * p < 0.05 vs. baseline diameter.

Figure 2.

Response of parietal pial arterioles to KCl in 2–3 months nonalcohol-fed (Control) (n=6) and alcohol-fed (Alcohol) (n=6) rats before and after suffusion with tempol (0.1 mM). Values are means $\pm$ SEM. * $P < 0.05$ vs. nonalcohol-fed rats. † $P < 0.05$ vs. response before suffusion with tempol.

Figure 3.

Response of parietal pial arterioles to KCl in 2–3 months nonalcohol-fed (Control) (n=6) and alcohol-fed (Alcohol) (n=7) rats before and after suffusion with apocynin (1 mM). Values are means $\pm$ SEM. * $P < 0.05$ vs. nonalcohol-fed rats. † $P < 0.05$ vs. response before suffusion with apocynin.

Figure 4.

Response of parietal pial arterioles to nitroglycerin in 2–3 months nonalcohol-fed (Control) (n=12) and alcohol-fed (Alcohol) (n=13) rats before and after suffusion with tempol (0.1 mM) or apocynin (1 mM). Values are means $\pm$ SEM.