Temporal effect of alcohol consumption on reactivity of pial arterioles: role of oxygen radicals

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Temporal effect of alcohol consumption on reactivity of pial arterioles: role of oxygen radicals. Am J Physiol Heart Circ Physiol 280: H992–H1001, 2001.—Chronic alcohol consumption reduces nitric oxide synthase-dependent responses of pial arterioles via mechanisms that remain uncertain. In addition, the temporal effects of alcohol on pial arterioles is unclear. Thus our goals were to examine the role of oxygen-derived free radicals in alcohol-induced impairment of cerebrovascular reactivity and the temporal effect of alcohol on reactivity of pial arterioles. Sprague-Dawley rats were pair-fed a liquid diet with or without alcohol for 2–3 wk, 2–3 mo, or 5–6 mo. We measured the in vivo diameter of pial arterioles in response to nitric oxide synthase-dependent dilators acetylcholine and ADP and the nitric oxide synthase-independent dilator nitroglycerin. In nonalcohol-fed rats, acetylcholine (1.0 and 10 mM) and ADP (10 and 100 μM) produced dose-related dilatation of pial arterioles. Whereas there was no difference in reactivity of arterioles to the agonists in rats fed the nonalcohol and alcohol diets for a period of 2–3 wk, there was a significant impairment in reactivity of arterioles to acetylcholine and ADP, but not nitroglycerin, in rats fed the alcohol diet for longer durations. We then found that treatment with superoxide dismutase did not alter baseline diameter of pial arterioles in nonalcohol-fed or alcohol-fed rats, but significantly improved impaired nitric oxide synthase-dependent dilatation of pial arterioles in alcohol-fed rats. Thus our findings suggest a temporal relationship in the effects of alcohol on reactivity of pial arterioles and that impaired nitric oxide synthase-dependent cerebral vasodilatation during chronic alcohol consumption may be related, in part, to enhanced release of oxygen-derived free radicals.

Sun, Hong, and William G. Mayhan. Temporal effect of alcohol consumption on reactivity of pial arterioles: role of oxygen radicals. Am J Physiol Heart Circ Physiol 280: H992–H1001, 2001.—Chronic alcohol consumption reduces nitric oxide synthase-dependent responses of pial arterioles via mechanisms that remain uncertain. In addition, the temporal effects of alcohol on pial arterioles is unclear. Thus our goals were to examine the role of oxygen-derived free radicals in alcohol-induced impairment of cerebrovascular reactivity and the temporal effect of alcohol on reactivity of pial arterioles. Sprague-Dawley rats were pair-fed a liquid diet with or without alcohol for 2–3 wk, 2–3 mo, or 5–6 mo. We measured the in vivo diameter of pial arterioles in response to nitric oxide synthase-dependent dilators acetylcholine and ADP and the nitric oxide synthase-independent dilator nitroglycerin. In nonalcohol-fed rats, acetylcholine (1.0 and 10 mM) and ADP (10 and 100 μM) produced dose-related dilatation of pial arterioles. Whereas there was no difference in reactivity of arterioles to the agonists in rats fed the nonalcohol and alcohol diets for a period of 2–3 wk, there was a significant impairment in reactivity of arterioles to acetylcholine and ADP, but not nitroglycerin, in rats fed the alcohol diet for longer durations. We then found that treatment with superoxide dismutase did not alter baseline diameter of pial arterioles in nonalcohol-fed or alcohol-fed rats, but significantly improved impaired nitric oxide synthase-dependent dilatation of pial arterioles in alcohol-fed rats. Thus our findings suggest a temporal relationship in the effects of alcohol on reactivity of pial arterioles and that impaired nitric oxide synthase-dependent cerebral vasodilatation during chronic alcohol consumption may be related, in part, to enhanced release of oxygen-derived free radicals.

CHRONIC ALCOHOL CONSUMPTION has been recognized as a major contributing factor in the pathogenesis of many cardiovascular diseases, including dilated cardiomyopathy, systemic hypertension, cardiac arrhythmias, and both hemorrhagic and ischemic stroke (5, 11, 16, 20). In addition, some investigators (20, 40, 43) have reported that acute alcohol exposure produces graded contractile responses in cerebral vessels and thus may contribute to the pathogenesis of stroke during binge drinking. In previous studies, we reported that dilatation of pial arterioles in response to agonists that stimulate the synthesis/release of nitric oxide is profoundly impaired during acute exposure to high concentrations of alcohol and during chronic (2–3 mo) alcohol consumption (22, 23). No information, however, is available regarding a possible temporal effect of chronic alcohol consumption on reactivity of cerebral blood vessels. Other investigators (17, 19, 37, 41) have demonstrated a temporal relationship in vascular reactivity during other disease states, and thus we speculated that the duration of exposure to alcohol might influence the degree of impairment of pial arterioles in response to nitric oxide synthase-dependent agonists. In addition, we reasoned that the duration of exposure to alcohol might influence cellular mechanisms that account for impaired responses of pial arterioles during alcohol consumption.

Mechanisms that contributed to impaired nitric oxide synthase-dependent dilatation of pial arterioles during acute exposure to alcohol and chronic alcohol consumption are not known. Several possible mechanisms may explain impaired responses of blood vessels during chronic consumption of alcohol. It is possible that alcohol consumption reduces the production of nitric oxide from endothelium and/or stimulates the release of an endothelium-derived contracting factor (34–36, 42). In addition, a number of studies have demonstrated that alcohol can stimulate lipid peroxidation through an increased formation of free radicals or by exhausting the antioxidant defense system, leading to oxidative stress in organ systems, including the brain (1, 29, 33). Increase formation of oxygen radicals could then directly damage the endothelium, affecting the synthesis/release of nitric oxide, and/or scavenge nitric oxide to alter vascular reactivity. Furthermore, some investigators (4, 40, 43) have shown that activation of endothelial nitric oxide synthase (eNOS) not only stimulates the release of nitric oxide, but may also stimulate the production of superoxide anion. Thus it is conceivable that chronic alcohol consumption may alter the balance between the production of nitric oxide and superoxide anion during receptor-mediator activation of eNOS.

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The first goal of this study was to examine a potential temporal effect of alcohol consumption on nitric oxide synthase-dependent and -independent responses of pial arterioles. Our hypothesis was that nitric oxide synthase-dependent dilatation of pial arterioles would be inversely related to the duration of exposure to the alcohol diet. Our second goal was to examine a potential cellular mechanism for the effects of chronic alcohol consumption on reactivity of pial arterioles. Our hypothesis was that impaired nitric oxide synthase-dependent dilatation of pial arterioles during alcohol consumption is related to the production of oxygen radicals to presumably inactivate nitric oxide.

METHODS

Experimental diets. We used male Sprague-Dawley rats in these studies. At about 2 mo of age (200–220 g body wt), the rats were randomly divided into one of six groups: three nonalcohol-fed groups and three alcohol-fed groups. To study the temporal effects of chronic alcohol consumption on the cerebral microcirculation and mechanisms contributing to impaired reactivity of pial arterioles during alcohol consumption, we pair-fed rats liquid diets for 2–3 wk, 2–3 mo, or 5–6 mo. The rats in the nonalcohol-fed groups were given a liquid diet that did not contain ethanol (Dyets, Bethlehem, PA). This diet is 1.0 kcal/ml, of which 35% are derived from fat, 47% are derived from carbohydrates, and 18% are derived from protein. Rats in the alcohol-fed groups were given a liquid diet (Dyets) containing ethanol. This diet is 1.0 kcal/ml, of which 35% are derived from fat, 11% are derived from carbohydrates, 18% are derived from protein, and 36% are derived from ethanol. The ethanol was gradually introduced into the diet over an 8-day period, as described previously (22, 24). Timing for microvascular studies was based on the duration that rats received the maximum concentration of ethanol. On the day of the experiment, the rat to be studied did not receive any diet. In the present study, this methodology produced a plasma alcohol concentration of 18 ± 2 mM, similar to what we previously reported (22). We measured the daily volume of diet consumed by the rats. The total daily volume of diet fed to the nonalcohol-fed rats was based on the daily consumption of diet by the alcohol-fed rats. Thus the daily consumption of diet was similar in the nonalcohol-fed and alcohol-fed rats.

Preparation of animals. Before we conducted the microvascular studies, rats were fed the nonalcohol diet or alcohol-containing diet for 2–3 wk, 2–3 mo, or 5–6 mo. On the day of the experiment, the rats were anesthetized with thiobutabarbital sodium (Inactin) (100 mg/kg body wt ip), and a tracheotomy was performed. The animals 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 plasma alcohol and thiobarbituric acid reactive substances (TBARS).

To visualize the microcirculation of the cerebrum, a craniectomy was prepared over the left parietal cortex (26). The cranial window was suffused with artificial cerebrospinal fluid (2 ml/min) bubbled continuously with 95% nitrogen and 5% carbon dioxide. The temperature of the suffusate was maintained at 37 ± 1°C. The cranial window was connected via a three-way valve to an infusion pump, which allowed for infusion of agonists and antagonists into the suffusate. This method, which we used previously (22, 24), maintained a constant temperature, pH, P CO2, and P O2 of the suffusate during infusion of drugs. Arterial blood gases were monitored and maintained within normal limits throughout the experimental period. Diameter of pial arterioles was measured using a video image shearing device (model 908, Instrumentation for Physiology and Medicine).

Experimental protocol. Cerebral vessels were superfused with artificial cerebrospinal fluid for 1 h before testing responses of arterioles to the agonists. Responses of pial arterioles were examined during superfusion of the agonists acetylcholine (1.0 and 10 μM) and ADP (10 and 100 μM), which presumably produce vasodilatation via the activation of eNOS and the release of nitric oxide. We also examined the responses of cerebral arterioles to nitroglycerin (0.1 and 1.0 μM), which dilates pial arterioles independently of nitric oxide synthase. To examine a temporal effect of alcohol consumption on reactivity of pial arterioles, we examined responses of arterioles to the agonists in rats fed the nonalcohol diet and the alcohol-containing diet for 2–3 wk, 2–3 mo, and 5–6 mo. To examine the potential role for oxygen radicals in impaired responses of pial arterioles during alcohol consumption, we initially examined responses of arterioles to the agonists, then we suffused the cranial window preparation with cerebrospinal fluid containing superoxide dismutase (SOD) (150 U/ml). One hour after starting the suffusion of SOD, and continuing for the duration of the experiment, we again examined responses of pial arterioles to the agonists.

Drugs were mixed in artificial cerebrospinal fluid and then superfused over the cerebral microcirculation. Application of agonists was randomized, and in each rat we studied responses of the largest pial arteriole exposed by the craniectomy. The diameter of pial arterioles was measured immediately before application of agonists and every minute for 5 min during application of agonists. Steady-state response to agonists was reached within 2 to 3 min after starting application of the agonist, and the diameter of pial arterioles returned to baseline within 3 to 5 min after application of the agonist was stopped.

Assay of TBARS. Lipid peroxides in the plasma of nonalcohol-fed and alcohol-fed rats were measured as described previously (1, 9, 30, 39). Arterial blood (0.05 ml) was withdrawn before the experiment and was added to 1 ml of heparinized saline. After centrifugation at 4,000 rpm for 10 min, 0.5 ml of supernatant was transferred to another centrifuge tube, and plasma lipids were isolated by precipitating with phosphotungstic acid, followed by the reaction with thiobarbituric acid. Fluorometric measurement of TBARS was made using a spectrophotofluorometer. The results were expressed as malonaldehyde (nmol/ml of blood), using as a standard the fluorescence intensity of the solution obtained by reacting 0.5 nmol of tetraethoxypropane with thiobarbituric acid.

Drugs. Acetylcholine chloride, ADP, and SOD (from bovine erythrocytes) were purchased from Sigma Chemical (St. Louis, MO). Nitroglycerin was purchased from Soltak Laboratories (Elk Grove Village, IL). Stock solutions of the agonists and antagonists were made in saline and dilutions were performed with the suffusate (artificial cerebrospinal fluid).

Statistical analysis. An unpaired t-test was used to compare values between nonalcohol-fed and alcohol-fed rats in response to the agonists. A Student’s paired t-test was used to compare values obtained before and after application of SOD. Values are means ± SE. A P value of 0.05 or less was considered to be significant.
RESULTS

Responses to agonists following 2–3 wk on alcohol diet. Baseline diameter of pial arterioles was $55 \pm 3\, \mu m$ in nonalcohol-fed rats and $49 \pm 3\, \mu m$ in rats fed the alcohol diet for 2–3 wk ($P > 0.05$). Body weight was $315 \pm 7\, g$ in nonalcohol-fed rats and $275 \pm 11\, g$ in alcohol-fed rats ($P > 0.05$). Acetylcholine, ADP, and nitroglycerin produced similar dose-related dilatation of pial arterioles in nonalcohol-fed rats and alcohol-fed rats (Fig. 1).

Responses to agonists following 2–3 mo on alcohol diet. Baseline diameter of pial arterioles was $36 \pm 1\, \mu m$ in nonalcohol-fed rats and $37 \pm 2\, \mu m$ in rats fed the alcohol diet for 2–3 mo ($P > 0.05$). Body weight in nonalcohol-fed rats was $389 \pm 7$ and $375 \pm 5\, g$ in alcohol-fed rats ($P > 0.05$). In rats fed the nonalcohol diet for 2–3 mo, acetylcholine (Fig. 2) and ADP (Fig. 3) produced dose-related dilatation of pial arterioles. However, responses of pial arterioles to acetylcholine (Fig. 2) and ADP (Fig. 3) were profoundly impaired in
rats fed the alcohol-containing diet for 2–3 mo. Dilatation of pial arterioles in response to nitroglycerin was similar in nonalcohol-fed and alcohol-fed rats (Fig. 4).

Topical application of SOD did not alter baseline diameter of pial arterioles in nonalcohol-fed and alcohol-fed rats (36 ± 6 vs. 36 ± 2 μm; *P > 0.05) and alcohol-fed rats (37 ± 2 vs. 39 ± 1 μm; †P > 0.05) rats. Thus there does not appear to be a basal effect of oxygen-derived free radicals on diameter of pial arterioles in nonalcohol-fed and alcohol-fed rats, although the concentration of TBARS in plasma was significantly elevated in alcohol-fed rats (Fig. 5). Topical application of SOD did not alter dilatation of pial arterioles in response to acetylcholine (Fig. 2), ADP (Fig. 3), or nitroglycerin (Fig. 4) in nonalcohol-fed rats. In contrast, topical application of SOD significantly improved dilatation of pial arterioles in response to acetylcholine (Fig. 2) and ADP (Fig. 3) in alcohol-fed rats. Topical application of SOD did not alter dilatation of the pial arterioles in response to nitroglycerin (Fig. 4) in alcohol-fed rats.

Responses to agonists following 5–6 mo on alcohol diet. Baseline diameter of pial arterioles was 48 ± 3 μm in nonalcohol-fed rats and 44 ± 3 μm in rats fed the alcohol diet for 5–6 mo (P > 0.05). Body weight in nonalcohol-fed rats was 534 ± 15 and 513 ± 12 g in alcohol-fed rats (P > 0.05). In rats fed the nonalcohol
diet for 5–6 mo, acetylcholine (Fig. 6) and ADP (Fig. 7) produced dose-related dilatation of pial arterioles. However, similar to that reported for rats fed the alcohol-containing diet for 2–3 mo, responses of pial arterioles to acetylcholine (Fig. 6) and ADP (Fig. 7) were profoundly impaired in rats fed the alcohol-containing diet for 5–6 mo. Dilatation of pial arterioles in response to nitroglycerin was similar in nonalcohol-fed and alcohol-fed rats (Fig. 8).

Topical application of SOD did not alter baseline diameter of pial arterioles in nonalcohol-fed (48 ± 3 μm, P > 0.05) and alcohol-fed (43 ± 3 μm, P > 0.05) rats. In addition, topical application of SOD did not alter dilatation of pial arterioles in response to acetylcholine (Fig. 6), ADP (Fig. 7), or nitroglycerin (Fig. 8) in nonalcohol-fed rats. In contrast, topical application of SOD significantly improved dilatation of pial arterioles in response to acetylcholine (Fig. 6) and ADP (Fig. 7) in alcohol-fed rats. Topical application of SOD did not alter dilatation of the pial arterioles in response to nitroglycerin (Fig. 8) in alcohol-fed rats.

**DISCUSSION**

There are three major new findings in this study. First, long-term (2–3 mo and 5–6 mo), but not short-term (2–3 wk), consumption of alcohol impairs nitric oxide synthase-dependent, but not -independent, dilatation of pial arterioles. Second, treatment of the cerebral microcirculation with SOD significantly improved, and in most cases restored, reactivity of pial arterioles in alcohol-fed rats toward that observed in nonalcohol-fed rats. Third, plasma levels of TBARS (an index of lipid peroxidation) are significantly increased in long-term, alcohol-fed rats.

Thus there appears to be a temporal effect, but not a progressive effect, of alcohol consumption on reactivity of pial arterioles to nitric oxide synthase-dependent agonists. In addition, we suggest that the mechanism of impaired nitric oxide synthase-dependent dilatation of pial arterioles during chronic alcohol consumption may be related to an increased release of oxygen-derived free radicals, presumably superoxide anion, which may serve to inactivate nitric oxide. We speculate that receptor-mediator activation of eNOS in alcohol-fed rats may produce an imbalance in the formation of nitric oxide and superoxide anion and thus may contribute to impaired nitric oxide synthase-dependent dilatation of pial arterioles.

**Consideration of methods.** To determine the effect of chronic alcohol consumption on nitric oxide synthase-dependent reactivity of pial arterioles, we examined...
responses to acetylcholine and ADP. We also examined responses of pial arterioles to nitroglycerin, which produces vasodilatation independently of nitric oxide synthase. Previous studies have shown that dilatation of pial arterioles in response to acetylcholine and ADP, but not nitrovasodilators, can be attenuated by application of enzymatic inhibitors of nitric oxide synthase (8, 18, 21). Thus dilatation of rat pial arterioles in response to acetylcholine and ADP is related to the synthesis/release of nitric oxide.

In a previous study (22), we reported that consumption of the alcohol-containing diet for a period of 2–3 mo impaired nitric oxide synthase-dependent dilatation of pial arterioles. In the present study, we were interested in determining whether there was a temporal effect of alcohol consumption on nitric oxide synthase-dependent and-independent responses of pial arterioles. Previous studies demonstrated a temporal relationship in vascular reactivity during other disease states (17, 19, 37, 41), and thus we speculated that short-term exposure to alcohol may not produce significant impairment in nitric oxide synthase-dependent cerebral vasodilatation. In contrast, we reasoned that more prolonged exposure to alcohol may produce more profound and potentially irreversible impairment in nitric oxide synthase-dependent reactivity of pial arterioles.

Fig. 7. Response of cerebral arterioles to ADP in 5–6 mo nonalcohol-fed rats (control) (n = 8) and 5–6 mo alcohol-fed rats (alcohol) (n = 8) before and after suffusion with SOD. Values are means ± SE. *P < 0.05 vs. nonalcohol-fed rats. †P < 0.05 vs. response before suffusion with SOD.

Fig. 8. Response of cerebral arterioles to nitroglycerin in 5–6 mo nonalcohol-fed rats (control) (n = 8) and 5–6 mo alcohol-fed rats (alcohol) (n = 8) before and after suffusion with SOD. Values are means ± SE.
Microcirculation. Acute application of SOD restored vasodilatation of pial arterioles. Thus we elected to examine the effects of feeding the alcohol-containing diet for three time periods (2–3 wk, 2–3 mo, 5–6 mo). We found that short-term exposure (2–3 wk) to the alcohol diet did not alter nitric oxide synthase-dependent or -independent responses of pial arterioles. In contrast, longer exposure to the alcohol-containing diet (2–3 mo and 5–6 mo) selectively impaired nitric oxide synthase-dependent dilatation of pial arterioles. However, there does not appear to be a progression in impaired nitric oxide synthase-dependent dilatation of pial arterioles during consumption of the alcohol-containing diet. There was a similar degree of impairment in nitric oxide synthase-dependent dilatation of pial arterioles in rats fed the alcohol-containing diet for 2–3 mo and 5–6 mo. Thus there appears to be a temporal, but not a progressive, impairment of nitric oxide synthase-dependent dilatation of pial arterioles during alcohol consumption. Although we suggest a temporal effect of alcohol on responses of pial arterioles, we cannot rule out the possibility that impaired responses of pial arterioles in the alcohol-fed rats may be related to possible influences of growth and development. It is conceivable that alcohol might influence vascular reactivity differently at distinct stages of growth and development. Thus it is possible that there is a temporal vulnerability of pial arterioles during exposure to alcohol.

In the present study, we found that baseline diameter of pial arterioles was different among rats on the diets for 2–3 mo and those on the diets for 2–3 wk and 5–6 mo. The explanation for this finding remains unclear. We know of no studies that have systematically examined the influence of age on baseline diameter of pial arterioles. Thus we presume this difference represents scientific variability. In any event, it is important to note that reactivity of pial arterioles in the rats on the diets for 2–3 mo was the same (in percent change in diameter) as that reported for rats on the diets for 2–3 wk and 5–6 mo. Although baseline diameter of pial arterioles was somewhat different in rats on the diets for 2–3 mo, interpretation of the data is not altered by this difference in baseline diameter.

To examine a potential role for oxygen radicals in impaired nitric oxide synthase-dependent vasodilation during alcohol consumption, we first measured lipid peroxidation levels in plasma (TBARS). We found that TBARS were modestly but significantly increased in long-term, alcohol-fed rats. This increase in TBARS appears to agree with that reported by Agar et al. (1). Although these investigators (1) did not examine levels of TBARS in plasma during alcohol consumption, they report a marked elevation of TBARS in tissue from the brain stem in alcohol-fed rats. Thus it is possible that if the brain is an important target organ for the effects of alcohol, then tissue levels of TBARS in the brain may be greater than that found in plasma. In addition to the measurement of TBARS, we examined a potential role of oxygen radicals in impaired nitric oxide synthase-dependent vasodilation during alcohol consumption by applying SOD to the cerebral microcirculation. Acute application of SOD restored impaired nitric oxide synthase-dependent, but did not alter nitric oxide synthase-independent vasodilatation during chronic alcohol consumption. In addition, topical application of SOD did not alter vasodilatation in nonalcohol-fed rats. Thus it appears that the effects of SOD are specific for nitric oxide synthase-dependent agonists and are specific for rats fed the alcohol-containing diet. The finding that acute superfusion with SOD significantly improved, and in some cases restored, impaired nitric oxide synthase-dependent vasodilatation during chronic alcohol consumption may be somewhat surprising. One might have predicted that, if oxygen radicals are produced chronically during alcohol consumption, then this might produce permanent damage to the endothelium to impair nitric oxide synthase-dependent dilatation. Thus we would not have predicted that acute superfusion with SOD would produce the dramatic effects on nitric oxide synthase-dependent vasodilatation observed in the present study. However, it is interesting to note that previous studies that examined the role of oxygen radicals in impaired responses of blood vessels during various disease states (7, 15, 25, 27) also have shown that acute treatment with scavengers of oxygen radicals restores nitric oxide synthase-dependent vasoreactivity. Thus results of the present study appear to be similar to what others have observed in other disease states. Furthermore, SOD did not alter vasodilatation in response to nitrovasodilators. Nitrovasodilators appear to produce relaxation of vascular smooth muscle via an intracellular enzymatic conversion to nitric oxide (3, 10). Because SOD did not alter responses to a nitrovasodilator in the present study and in previous studies (7, 15, 25, 27), we suggest that oxygen radicals may act extracellularly to inhibit the actions of nitric oxide.

Consideration of previous studies. Previous studies have examined the effects of acute and chronic alcohol exposure on nitric oxide synthase-dependent response of large and small blood vessels. However, mechanisms that account for the effects of alcohol on vascular reactivity remain unclear. A study using the isolated, perfused rat mesenteric artery preparation showed that acute infusion of alcohol impaired endothelium-dependent dilatation in response to acetylcholine and ATP (6). In addition, other investigators (12, 13) have shown that acute exposure of rat aorta to alcohol impaired endothelium-dependent dilatation in response to acetylcholine and ATP. In contrast, dilatation in response to endothelium-independent dilators, papaverine, or sodium nitroprusside, was not altered by acute alcohol exposure (6, 12, 13). Furthermore, we previously reported that acute exposure of pial arterioles to alcohol (80 and 100 mM) impaired nitric oxide synthase-dependent, but not -independent, dilatation (23). Thus it appears that exposure of blood vessels to alcohol suppress nitric oxide synthase-dependent dilatation of large peripheral and small cerebral blood vessels.

We were the first to examine the effect of chronic alcohol consumption on nitric oxide synthase-dependent dilatation of rat pial arterioles (22). We found that dilatation of pial arterioles in response to nitric oxide...
synthase-dependent, but not -independent, dilators was impaired in rats fed an alcohol-containing diet for 2–3 mo. However, mechanisms that accounted for the effects of alcohol on pial arterioles was not examined in our previous study (22). In the present study, we report that SOD significantly improves impaired responses of pial arterioles to nitric oxide synthase-dependent agonists during chronic alcohol consumption. Thus we suggest that oxygen radical formation plays a key role in impaired responses of pial arterioles during chronic alcohol consumption.

Mechanisms of altered nitric oxide synthase-dependent responses. Mechanisms that contribute to altered vascular reactivity during chronic alcohol consumption are complex. First, because responses of blood vessels to nitrovasodilators are unaffected by acute or chronic alcohol exposure, one might conclude that alcohol produces morphological abnormalities of the endothelium, which may directly affect endothelial function, i.e., synthesis/release of nitric oxide. In fact, one study has shown that intravenous infusion of ethanol in rabbits decreased exhaled nitric oxide (36).

Second, it is possible that alcohol stimulates lipid peroxidation through the formation of free radicals and/or exhausts the antioxidant defense system, leading to oxidative stress in the brain. This increase in the formation of oxygen radicals could directly damage the endothelium affecting the synthesis/release of nitric oxide, and/or scavenge nitric oxide to affect vascular reactivity. Investigators have shown that chronic alcohol consumption causes oxidative insults to many organ systems, including the brain (1, 29, 33). Motolu et al. (29) found that chronic alcohol consumption led to an induction of cytochrome P-450 and the alcohol-inducible form of cytochrome P-450 (CYP2E1; a potent generator of oxygen radicals) and produced a decrease in GSH/GSSG ratio in rat brain. In addition, Omadeo-Sale et al. (33) reported that chronic alcohol consumption produced an increase in lipid peroxidation and a decrease in activity of Cu/Zn SOD in rat microsomes. Moreover, a recent study reported that chronic alcohol consumption produced a dose-related increase in TBARS, and a dose-related decrease in glutathione levels in the brain stem of rats (1). In the present study we found that TBARS were significantly increased in long-term alcohol-fed rats. Although the precise source of lipid peroxidation cannot be determined by the present study, we suggest that increased lipid peroxidation could influence nitric oxide synthase-dependent vasodilatation.

Third, it is possible that impaired nitric oxide synthase-dependent vasodilatation during chronic alcohol consumption may be related to alcohol-related metabolic mechanisms. The initial step of alcohol oxidative metabolism is the formation of acetaldehyde. Acetaldehyde and metabolites of acetaldehyde may be toxic to endothelium and account for impaired nitric oxide synthase-dependent dilatation of blood vessels during alcohol consumption.

Fourth, some studies have shown that activation of eNOS not only stimulates the release of nitric oxide, but may also stimulate the production of superoxide anion (4, 40, 43). Thus it is conceivable that there is an imbalance in the production of nitric oxide/oxygen radicals during chronic alcohol consumption. An excess in the receptor-mediated release of oxygen radicals by eNOS could inactive nitric oxide released in response to agonist-induced stimulation and thus account for impaired nitric oxide synthase-dependent dilatation of pial arterioles in alcohol-fed rats. In the present study, we speculated that if oxygen radicals were produced chronically during alcohol consumption, then we might see a change in baseline diameter of pial arterioles in alcohol-fed rats. However, we did not see a change in baseline diameter of arterioles between nonalcohol-fed and alcohol-fed rats. Furthermore, we reasoned that, if oxygen radicals were elevated by chronic alcohol consumption, then superfusion with SOD might alter the diameter of pial arterioles in alcohol-fed rats. However, we did not observe an effect of SOD on the baseline diameter of pial arterioles in alcohol-fed rats. At least two possibilities exist. First, it is possible that the chronic production of low levels of oxygen radicals, which do not affect baseline diameter of pial arterioles, may influence vasoreactivity in response to agonists that stimulate an increase in nitric oxide synthesis/release. Second, it is possible that chronic alcohol consumption alters receptor-mediated synthesis/release of nitric oxide/oxygen radicals via eNOS and this alteration accounts for impaired reactivity of pial arterioles in rats fed the alcohol-containing diet.

In summary, we examined the effect of chronic alcohol consumption on reactivity of pial arterioles. We found a temporal relationship between alcohol consumption and impaired reactivity of pial arterioles. In addition, we found that inhibition of oxygen radicals using SOD significantly increased nitric oxide synthase-dependent dilatation of pial arterioles in the alcohol-fed rats. We believe that impairment of cerebral vasodilation during chronic alcohol consumption is significant regarding the regulation of cerebral blood flow. Because tissue perfusion varies with the cube of the diameter of a vessel (28), modest to moderate changes in diameter of pial arterioles can greatly alter local cerebral blood flow. Thus alterations in reactivity of pial arterioles observed during chronic alcohol consumption may be important for the regulation of local cerebral blood flow and altered response of pial arterioles during chronic alcohol consumption may produce detrimental effects on the brain. Many studies have shown that cerebral blood flow is decreased in chronic alcoholics (31, 32, 38) and that alcohol consumption is a risk factor for the pathogenesis of stroke (14, 20). Thus we speculate that impaired responses of cerebral blood vessels during alcohol consumption may have important implications for the pathogenesis of ischemic and hemorrhagic stroke observed in chronic alcoholics.

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