

Acute Ethanol Effects on Focal Cerebral Ischemia in Fasted Rats

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The effects of acute ethanol intoxication were investigated in a rat model of unilateral middle cerebral artery occlusion. Groups of 5 to 8 male Sprague-Dawley rats were subjected to 4 hr of left middle cerebral artery occlusion. All groups were deprived of food overnight and were pretreated intraperitoneally with 5% dextrose solution (10 ml/kg), 20% ethyl alcohol in 5% dextrose solution (2 g/kg), or 30% ethyl alcohol in a 5% dextrose solution (3 g/kg) 1 hr before middle cerebral artery occlusion. Regional cerebral blood flow during ipsilateral occlusion was ~9.1 to 10% of baseline in all groups. The mean % brain water content in control, 2 g/kg ethanol-treated groups, and 3 g/kg ethanol-treated groups were: in the ischemic core—81.6, 81.2, and 82.4; intermediate zone—80.5, 80.6, and 81.7; and outer zone—79.7, 79.7, and 80.8, respectively. Brain Na⁺ and K⁺ content in the three groups was related to water content, but much greater with ethanol pretreatment. The water content of the intermediate zones in the 3 g/kg ethanol-treated animals was significantly greater than in the control ($p < 0.01$ and 0.001) and the 2 g/kg ethanol-treated groups. One-way analysis of variance indicated a significant dose-effect relationship in which the lower dose of ethanol tended to reduce ischemic core water content, and the larger dose increased ischemic core water, compared with the control. None of the overnight fasted groups had any significant hyperglycemia. The group given 3 g/kg ip ethanol 1 hr before had exacerbated edema formation with a mean whole blood level of ethanol of ~230 mg/dl. The neurotoxic effects of high concentrations of ethanol were unrelated to any change in plasma glucose concentrations.

Key Words: Ethanol, Ethyl Alcohol, Hyperglycemia, Insulin, Ischemia.

SOME STUDIES using different experimental models and species have found that ethanol worsens brain and spinal cord injury, as well as its cardiovascular and respiratory consequences, whereas others have reported that ethanol reduces lesion size and functional recovery time. Flamm et al.¹ found that ethanol potentiates central nervous system trauma in anesthetized cats. Albin and Bunge² observed that ethanol increased brain lesion volume in both normotensive and hypotensive anesthetized dogs, compared with control. Dimethyl sulfoxide (DMSO) markedly attenuated the untoward effects of ethanol. These

investigators postulated that ethanol intermediate metabolites produced hydroxyl-free radicals which, in combination with neuronal tissue damage, potentiated the trauma that was reduced by free radical scavenging with DMSO. Bunge et al.³ found that animals first intoxicated with ethanol followed by a focal ischemic injury formed 3.1 and 3.4% more free radicals in the lesioned brain tissue than in the controls. They concluded that free radical formation and the resulting focal ischemia are enhanced in the presence of ethanol, compared with control ischemic brain tissue.

Franco et al.⁴ found that ethanol pretreated diethyl ether anesthetized Swiss Webster mice subjected to head trauma died more quickly than control mice not given ethanol. Zink and Feustel⁵ studied the effects of ethanol on brain injury and cerebral blood flow in a percussion model of traumatic injury in anesthetized pigs. These investigators found that ethanol intoxication produced significant hemodynamic and respiratory changes that had a deleterious effect on outcome and mortality after brain injury. Subsequently, Zink et al.⁶ extended their studies using the same traumatic brain injury model. Ethanol led to significant impairment of respiratory control and contributed to the extent of brain injury in the intoxicated animals. These animal studies are in agreement that ethanol intoxication in acutely brain injured humans leads to more severe injuries and a higher mortality rate.⁷⁻¹⁰ Kelly et al.¹¹ reported paradoxical effects of acute ethanol in experimental brain injury in Sprague-Dawley rats. Low to moderate ethanol concentrations reduced lesion size and increased functional recovery following traumatic brain injury, but high concentrations increased lethality.

The goal of the present research project was to determine the effects of acute ethanol in focal cerebral ischemia in rats in a highly reproducible model of middle cerebral artery occlusion (MCAO).^{12,13} The experiments were designed to mimic a human scenario of two ethanol severely intoxicated males and one male teetotaler, who fasted overnight except for a small amount of a sugar containing drink in the early morning. All had an identical MCAO stroke 1 hr later. The question to be answered was does ethanol affect the amount of brain edema when examined 4 hr later? The working hypothesis of this research was that large doses of ethanol are neurotoxic and worsen brain edema following MCAO.

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MATERIALS AND METHODS

Experimental Protocol

This study was approved by the University of Michigan Committee on the Use and Care of Animals. Adult male Sprague-Dawley rats weighing 230 to 300 g (Charles River) were randomly divided into five groups of MCAO for 4 hr. They were on a 7:00 AM to 7:00 PM light and 7:00 PM to 7:00 AM dark cycle on a standard rodent diet and water ad libitum. All animals were deprived of food overnight. They were pretreated 60 min before MCAO the next morning. The experimental groups were treated 60 min prior to MCAO as follows. Group 1 was the control. These animals received 5% dextrose solution (10 ml/kg, ip). Group 2 received 20% ethanol in 5% dextrose solution in a dose of 2 g/kg ip. Group 3 received 30% ethanol in 5% dextrose solution in a dose of 3 g/kg ip. Group 4 received 3 g/kg ip ethanol in 5% dextrose and were used to measure plasma ethanol levels. Group 5 were either control, 2, or 3 g/kg ethanol-treated animals used for magnetic resonance imaging (MRI) studies. During all experiments, physiological parameters were monitored and controlled. Local cerebral blood flow (CBF) was measured through the surface of the cortex using a Laser doppler flow monitor equipped with a small caliber probe of 0.7 mm diameter. Rectal temperature was measured by a thermometer (YSI, model 73A, Yellow Springs Instrument Co.). The temperature was carefully regulated using a heating lamp and heating pad to maintain 37°C. After each experiment, animals were sacrificed and brain samples measured for water and ion content. Ethanol was obtained from the University of Michigan Hospital Pharmacy.

Rat MCAO Suture Model

Anesthesia was induced by inhalation with 5% isoflurane in 0.1 liter/min O₂ and 0.8 liters/min air. Following tracheal intubation, the lungs were mechanically ventilated to maintain the PaCO₂ at ~40 mm Hg. Anesthesia was continued with 1 to 2.5% isoflurane. The femoral artery was cannulated with PE-50 tubing to allow continuous monitoring of arterial blood pressure and sampling of arterial gases, blood pH, and blood glucose. Blood pressure was maintained above 90 mm Hg by adjusting the isoflurane concentration. MCAO was produced as described previously.^{12,13} Briefly, under an operating microscope, the left common carotid artery (CCA) was exposed through a midline incision. Branches of the epidural catchment area (ECA) were isolated and coagulated along with the terminal lingual and maxillary artery branches. The internal carotid artery (ICA) was then isolated and its extracranial branch, the pterygopalatine artery, was ligated close to its origin. ICA remained patent. A 3-cm length of 3-0 nylon suture with a slightly enlarged and rounded tip was introduced into the transected lumen of the ECA and gently advanced from the ECA into the ICA. The distance from the tip of the suture to the bifurcation of the CCA was 19 to 20 mm in these rats.

CBF Measurement

A BPM2 laser Doppler flowmeter (Vasamedics, Inc.) was used for the CBF measurements in rats. Laser doppler flow was determined in two cortical sites. Point A was placed 7 mm lateral in the contralateral hemisphere, and point B was placed 7 mm lateral in the ipsilateral ischemic hemisphere. Both sites were 1.5 posterior to the bregma. After mounting the rat in a stereotaxic frame, the skull was exposed through a midline skin incision. Two partial holes were drilled such that a thin bony layer could be carefully removed to prevent injury to the cortex and maintain dura integrity. The probe was held in a micromanipulator and stereotactically advanced to gently touch the intact dura matter to obtain a clear optic medium between the flow probe and the cortex. To maintain brain temperature at 37° to 37.5°C, warmed 0.9% NaCl was slowly perfused around the probe during the experiment. Ten minutes of stable baseline flow readings were obtained prior to occlusion of the sites described previously. Then, MCAO was produced and the digital display of CBF was recorded. CBF values were calculated and expressed as percentage of baseline values (ml/100 g/min). If CBF increased over an arbitrary criterion of 35

ml/100 g/min during occlusion, the animal was excluded from the study. Laser Doppler flowmetry is generally not considered a very precise method of measuring small changes in CBF in ml/100 g/min. However, it is essential to determine if a MCAO was present and whether it changed markedly during the experimental period of ischemia.

Water, Na⁺, K⁺, and Cl⁻ Content

Samples were removed using 7 mm and 10 mm cork borers from the center, intermediate, and outer zones of the ischemic cerebral cortex and the corresponding areas of the contralateral nonischemic cortex using the same anatomical zones as illustrated in the publications of Betz and Coester¹⁴ and Martz et al.¹⁵ The tissue samples were weighed with 0.0001 mg precision to obtain each wet weight (*W*). The samples were then dried in a gravity oven (Blue M Electric Co.) at 100°C for 24 hr and reweighed to obtain their dry weight (*D*). The water content was expressed as % wet weight and was calculated as $(W - D)/W \times 100$. The dehydrated section was digested in 1 ml of 1 N nitric acid for 1 week. Then, a 0.2 ml aliquot was removed and diluted to 2 ml with deionized water and 3 mM CsCl₂ solution. The Na⁺ and K⁺ contents were measured with a sample of this solution by atomic absorption spectroscopy (IL943TM Automatic Flame Photometer, Instrumentation Laboratory, Inc.). Flame conditions and detection wavelengths were optimized for sensitivity and linearity. The Cl⁻ content was measured by a digital chloridometer (Haakebuchler Instruments, Inc.).

MRI

Multiple MRI of the rat brains were obtained beginning 3 hr and ending 4 hr after MCAO. Standard T₂-weighted MRI was performed on a Spectroscopy Imaging Systems Corporation unit equipped with a 7.0 Tesla, 18.3 cm horizontal bore magnet (300-MHz proton frequency). A 4-cm diameter birdcage radiofrequency coil was used for imaging the head of each rat. Fast gradient echo magnetic resonance images were obtained in orthogonal planes to confirm desired positioning. Acquisition parameters for T₂-weighted images were TR/TE = 3500/60; 128*128 matrix. The field of view = 30 mm. Two excitations were averaged. Twenty-five 0.5 mm coronal slices with 0.8 mm center-to-center spacing were obtained.

Plasma Ethanol and Glucose

Plasma ethanol was assayed using gas chromatography-flame ionization detection as described by Wagner and Patel.¹⁶ Plasma glucose was determined using a Glucometer II (Miles Laboratories, Inc.).

Statistical Analysis

All of the data were expressed as the mean ± SEM. Statistical differences among groups were determined using ANOVA, the Tukey-Kramer multiple comparison, and the Dunnett *t* tests. Probability values of <5% were considered significant.

RESULTS

Physiological and Pharmacological Parameters

The physiological parameters for each group at the end of 4 hr of MCAO are given in Table 1. Animals were excluded from this study if their physiological parameters were not in the normal range during the experiment. ANOVA failed to reveal a difference between the groups in any parameter. All animals had normal pre- and postischemia blood gases (pO₂, pCO₂, and HCO₃⁻), pH, hematocrit, and plasma osmolarity. The rectal temperature of each animal was monitored and maintained about 37.5°C as described. Mean arterial blood pressure was normal before

and after MCAO, with no difference between the various groups. The variation in plasma glucose within the treatment groups was surprisingly large and, therefore, no significant differences between groups were obtained. Inasmuch as the control group did not receive equicaloric treatments equivalent to 2 or 3 g/kg of ethanol, one would have expected a trend for the control group to have the lowest mean glucose level and not between the 2 and 3 g/kg treated groups. Group 3 rats given 3 g/kg of ethanol ip had a mean \pm SE plasma ethanol level before injection of 0, and 1 hr later of 285 ± 20 mg/dl. The latter is the equivalent of ~ 0.23 gm% total blood alcohol concentration of an intoxicated human, because whole blood alcohol concentrations are $\sim 80\%$ of plasma concentrations.

CBF

Introduction of the suture to occlude the blood supply to the territory of the left middle cerebral artery territory produced an equal fall in relative surface blood flow in all groups (Table 2). The mean percentage of baseline CBF in the contralateral normal hemisphere was comparable, remaining $\sim 100\%$ in all of the groups. During MCAO, the level of CBF in the ipsilateral hemisphere was reduced 9 to 10% of baseline in each group. There were no significant differences among the ischemic groups.

Water and Ion Content

The changes in water content in the core, intermediate, and outer zones of both ischemic and nonischemic cortex of all groups are summarized in Table 3. A one-way analysis of variance indicated that the variation among means for the ischemic cores is statistically significant ($F = 7.42, p = 0.006$). The Tukey-Kramer multiple comparison test indi-

Table 3. Water and Ion Concentration after MCAO

	Control	Ethanol (2 g/kg)	Ethanol (3 g/kg)
H₂O			
Ipsilateral			
Core	81.6 \pm 0.5	81.2 \pm 0.4	82.4 \pm 0.4**
Inner zone	80.5 \pm 0.4	80.6 \pm 0.3	81.7 \pm 0.2**
Outer zone	79.7 \pm 0.2	79.7 \pm 0.2	80.8 \pm 0.3***
Contralateral			
Core	78.7 \pm 0.3	78.9 \pm 0.1	78.5 \pm 0.2
Inner zone	79.1 \pm 0.2	79.6 \pm 0.2	79.3 \pm 0.2
Outer zone	79.2 \pm 0.3	79.6 \pm 0.2	79.3 \pm 0.1
Na⁺			
Ipsilateral			
Core	386 \pm 41	337 \pm 23	425 \pm 22*
Inner zone	272 \pm 30	252 \pm 13	315 \pm 15*
Outer zone	205 \pm 7	191 \pm 12	205 \pm 3
Contralateral			
Core	188 \pm 5	176 \pm 6	190 \pm 3
Inner zone	195 \pm 10	190 \pm 16	197 \pm 4
Outer zone	194 \pm 5	191 \pm 2	200 \pm 3
K⁺			
Ipsilateral			
Core	356 \pm 26	370 \pm 34	332 \pm 15*
Inner zone	471 \pm 29	448 \pm 43	443 \pm 11*
Outer zone	551 \pm 18	500 \pm 36	518 \pm 15
Contralateral			
Core	518 \pm 17	465 \pm 13	504 \pm 7
Inner zone	528 \pm 27	495 \pm 47	522 \pm 10
Outer zone	543 \pm 16	485 \pm 4	523 \pm 9
Cl⁻			
Ipsilateral			
Core	180 \pm 13	131 \pm 16	198 \pm 10*
Inner zone	186 \pm 17	146 \pm 13	176 \pm 8*
Outer zone	177 \pm 3	141 \pm 14	156 \pm 7
Contralateral			
Core	173 \pm 11	142 \pm 3	148 \pm 3
Inner zone	194 \pm 23	140 \pm 8	153 \pm 4
Outer zone	186 \pm 11	160 \pm 6	164 \pm 3

Data are the means \pm SEM; $n = 5-8$ rats in each group. p values are as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared with the control group.

Table 1. Physiological Parameters during MCAO

	Control	Ethanol (2 g/kg)	Ethanol (3 g)
Mean blood pressure (mm Hg)	101 \pm 7	96 \pm 2	101 \pm 3
Temperature ($^{\circ}$ C)	36.8 \pm 0.4	37.3 \pm 0.1	37.5 \pm 0.0
pH	7.44 \pm 0.06	7.46 \pm 0.03	7.38 \pm 0.02
pCO ₂ (mm Hg)	42 \pm 8	37 \pm 3	45 \pm 2
pO ₂ (mm Hg)	119 \pm 32	144 \pm 5	111 \pm 5
HCO ₃ (mmol/liter)	28 \pm 1	27 \pm 1	27 \pm 1
Hematocrit	0.4 \pm 0.0	0.4 \pm 0.0	0.5 \pm 0.0
Glucose (mg/dl)	131 \pm 21	104 \pm 6	154 \pm 23

Data are the means \pm SEM; $n = 5-8$ rats in each group after 4 hr of MCAO. All groups were fasted overnight in this and all subsequent tables and figures.

Table 2. Mean % Surface CBF following 4 hr of MCAO

Hemisphere	Control	Ethanol (2 g/kg)	Ethanol (3 g/kg)
Before MCAO			
Right	100 \pm 0	100 \pm 0	100 \pm 0
Left	100 \pm 0	100 \pm 0	100 \pm 0
After MCAO			
Right	99 \pm 2	96 \pm 5	99 \pm 3
Left	10 \pm 1	9 \pm 1	9 \pm 1

Values are % of baseline CBF as mean \pm SE; $n = 5-8$ rats in each group.

cated that the difference between the ischemic core for 2 g/kg (a trend lower than control) and 3 g/kg (a trend greater than control) is statistically significant ($p < 0.01$). Similar one-way analyses of variance for the intermediate and outer ischemic penumbra indicated that the variation among means is also statistically significant ($F = 8.72, p = 0.0035$; $F = 22.81, p = 0.0001$, respectively). A dose of 3 g/kg of ethanol increased the water content of the intermediate ($p < 0.01$), as well as the outer zones ($p < 0.001$) above control levels. However, the 2 g/kg ethanol water content did not differ from control MCAO water content in both penumbral regions. In all instances, the differences between 2 and 3 g/kg of ethanol were highly significant ($p < 0.001$ to < 0.01) using the Tukey-Kramer multiple comparison test. The increase in water of the ischemic brain tissue was accompanied by comparable shifts in Na⁺ and K⁺. After ethanol, brain Cl⁻ concentrations on the ischemic hemisphere tended to decrease. However, the mean Cl⁻ increase in the ischemic core following 3 g/kg is unexpected and needs to be confirmed in additional studies.

MRI

Magnetic resonance T_2 -weighted images showed an increase of signal intensity in the ischemic hemisphere in the rat treated with ethanol (3 g/kg body weight, ip) that was well localized (Fig. 1). However, there were no clear lesion borders in the 2 g/kg ethanol-treated and untreated control rats. The volume of infarct area in the 3 g/kg ethanol-treated rat was larger than in the 2 g/kg ethanol-treated and untreated control rats consistent, in part, with the quantitative data described previously. However, the MRI of the 3 g/kg rat showed a much larger volume of edema than that listed in Table 3.

DISCUSSION

This research involves laboratory rats. Therefore, a qualitative species difference from humans may exist. This possibility is relatively remote because this rat study is totally consistent with the neurotoxic effects of ethanol in human brain trauma and injury. So what is new? In the first place, we designed a controlled study of ethanol in a rat MCAO model that can be used to determine the mechanisms of ethanol neurotoxicity in vivo. Secondly, by using fasting rats, it was shown that a large dose of ethanol does not produce hyperglycemia. We have previously reported that moderate doses of ethanol in nonfasting rats produce hyperglycemia and enhanced neurotoxicity reversed by insulin.¹⁷ With a larger intoxicating dose of ethanol, enhanced

neurotoxicity was observed in normoglycemic fasting rats. Hence, hyperglycemia was ruled out as a possible mechanism of large dose ethanol neurotoxicity. The dose of ethanol that enhanced brain edema produced blood levels equivalent to a drunken human. A confounding variable is that a mixture of isoflurane, oxygen, and air were used to produce general anesthesia for the surgical procedure of MCAO. Thus, one is studying the effects of ethanol plus general anesthesia, compared with the effects of ethanol alone on brain ischemia. General anesthesia must be used for ethical reasons. Until more reliable methods are developed for producing MCAO without anesthesia, this variable must be present in research of this type. Depth of anesthesia (with and without ethanol) was closely monitored using arterial blood pressure, relief of surgical pain as noted by reflex motor movements, normal arterial blood gases, normal body temperature, etc.

Fasting rats given a large dose (3 g/kg, ip) of ethanol prior to induction of ischemia had more severe brain edema, compared with the control and the 2 g/kg ethanol-treated animals. An even lower dose of ethanol needs to be studied in the future to pursue the possibility it may be neuroprotective as observed by Kelly et al.¹¹ The very small amount of 5% glucose used as the vehicle solvent for the ethanol had no apparent effect on plasma glucose levels, inasmuch as all three groups of animals were normoglycemic. The mechanism of the deleterious effect of a whole blood alcohol concentration of ~ 0.23 g% in humans remains to be clarified. Such an ethanol level is frequently seen in clinically intoxicated patients with concomitant brain injury or ischemia. It can be argued that a whole blood alcohol level of 0.23 g% is so large in humans that this research in rats is irrelevant to most alcohol-intoxicated people. The minimum lethal whole blood concentration of ethanol in nontolerant humans is reported to be ~ 350 mg/dl.¹⁸ A 70 kg adult male will obtain a whole blood ethanol concentration of ~ 25 mg/dl, with an alcoholic drink equivalent that contains ~ 15 g of pure ethanol. Thus, $350/25 = 14$. Therefore, $15 \text{ g} \times 14 = 210 \text{ g}$ divided by $70 \text{ kg} = 3.0 \text{ g/kg}$. This means that the minimum lethal dose of ethanol taken orally by human nontolerant males is ~ 3.0 g/kg. On the other hand, the minimum lethal dose of ethanol given intraperitoneally to rats is 5 g/kg.¹⁹ The dose of 3 g/kg of ethanol given intraperitoneally to the rats in this study is, therefore, $3/5$ or 60% of the minimum rat lethal dose, whereas 3 g/kg orally is a minimum lethal dose in man. One can conclude that the rat is more resistant to the lethal effects of ethanol than man. Therefore, the concentration-effect relationship of ethanol in vivo in rats cannot be directly applied to humans. What is clear is that high concentrations of ethanol can make brain edema worse in rats with an MCAO in a controlled study; this finding completely supports the published human data. Most high-dose ethanol consumption occurs among chronic abusers and, therefore, chronic ethanol studies are indicated in the future. Furthermore, the 2 g/kg dose of ethanol showed a

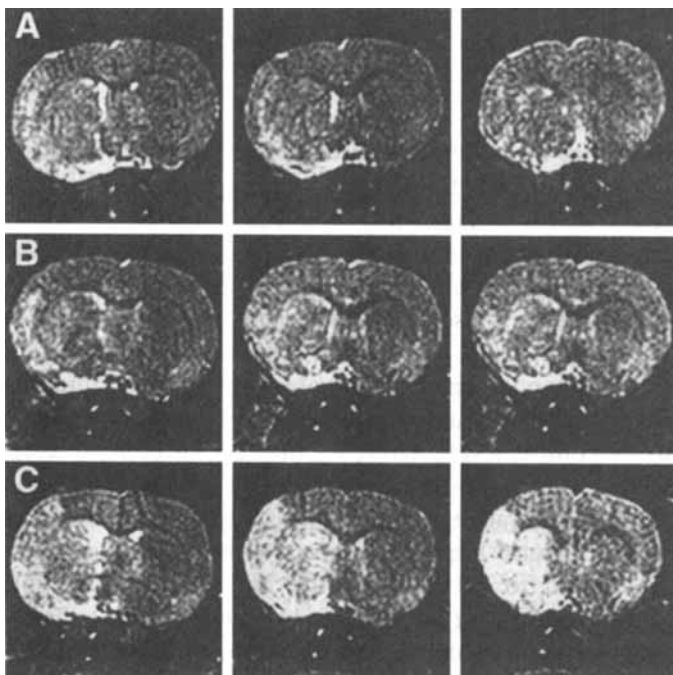


Fig. 1. Magnetic resonance T_2 -weighted images showing the deleterious effects of a large dose of ethanol on ischemic brain injury. The brains of three different MCAO rats are shown [(A) vehicle control, (B) 2 g/kg ethanol, and (C) 3 g/kg ethanol given ip 1 hr before left MCAO]. (Left to right) Posterior to anterior coronal slices of the same animal. Note the larger lesion (whiter area) in the right hemisphere of rat brain (C). For clarity, the lesion areas are outlined as illustrated.

definite trend for decreased ischemic core edema, indicating a possible neuroprotective effect. This is especially important. Future studies must also use much smaller doses of ethanol than used in this study.

Altura et al.²⁰ reported that ethanol in concentrations of 10 to 500 mg/dl produced graded contractile responses in rat cerebral arterioles and venules *in vivo* and in isolated canine basilar and middle cerebral arteries. Two calcium ion channel antagonists, nimodipine and verapamil, antagonized ethanol induced cerebrovasospasm. Ema et al.²¹ found that magnesium sulfate also prevented ethanol-induced spasm of rat cerebral vessels. Interestingly, the vasodilator effect of magnesium sulfate was greater in male than female rats. Zhang et al.²² studied the ethanol-induced contractions in concentrations of 8 to 570 nM in cerebral arteries from dogs, sheep, piglets, and baboons. The middle cerebral arteries were the most sensitive to ethanol-induced contractions. No gender differences were found in ethanol treated canine cerebral arteries. Ethanol produced vasoconstriction in such arteries in the presence or absence of endothelial cells. An absence of extracellular Mg^{2+} ion enhanced, and removal of extracellular Ca^{2+} ion reduced, ethanol-induced cerebral artery vasoconstriction. In addition, caffeine abolished ethanol cerebral artery vasoconstriction. It is well known that *in vitro* and *in vivo* open and closed window pial blood vessels show different vascular responses. Mayhan and Didion²³ found that high concentrations of ethanol *in vivo* in rats impair cerebral arteriole dilatation to agonists that stimulate either the synthesis or release of nitric oxide. These findings suggest several promising potential antagonists of ethanol neurotoxicity *in vivo*. Obviously, ethanol-induced cerebral artery vasospasm *in vivo* would impact on eventual neurological outcome. Another postulated mechanism that may be involved in the deleterious effects of large doses of ethanol is reactive oxygen species. Bunegin et al.³ reported increased free radical formation following ethanol intoxication in dogs. The present rat ischemia model of MCAO would also be useful to pursue this possible mechanism of ethanol neurotoxicity. Further research using scavengers of oxygen radicals should be done to determine if they prevent ethanol-induced increase in brain damage.

A clear distinction should be made between traumatic brain injury and stroke in relationship to ethanol. Much of the literature related to humans involves ethanol and brain trauma, as described in the introduction. There is evidence that excessive ethanol use increases the risk of strokes, whereas light to moderate ethanol ingestion may be neuroprotective.²⁴⁻²⁷ Hemorrhagic strokes are most commonly associated with alcoholism and alcohol abuse. In the present study, an ischemic stroke model was used because of its reproducibility, in contrast to a hemorrhagic stroke model.

Finally, this study in rats supports observations in humans and animals that MRI is useful in observing the evolution of an ischemic brain injury. However, the appar-

ent size of the MRI abnormalities shown in Fig. 1 is much larger than the quantified edema data in Table 3, indicating a discrepancy that must be studied further in a larger number of animals comparing *in vitro* and *in vivo* results. In the present study, standard T_2 -weighted MRI performed 3 to 4 hr post-MCAO was sufficient to document the spatial extent of the ischemic changes. Whether these represent a temporary or permanent brain injury needs to be documented. Additional MRI methods are applicable for yet earlier stages of vascular changes. In particular, cerebral blood volume-dependent MRI and water diffusion-weighted MRI offer the capabilities to visualize regions of reduced perfusion and tissue damage before changes are apparent on standard T_2 -weighted MRI.²⁸⁻³¹ These latter reports, especially by Basser et al.,²⁸ Moseley et al.,²⁹ and Sorensen et al.³⁰ emphasize the utility of diffusion imaging as being more sensitive, in contrast to the standard method we used. However, it is clear that the MRI method used in the present study was able to document visually dramatic regional brain changes that indicate that large doses of ethanol enhance brain edema following MCAO. The regional and temporal detail afforded by all MRI methodologies will help to better identify the ischemic injury, as well as to facilitate development of new neuroprotective agents, and for staging of strokes in patient management.³¹ One can use the present animal model of MCAO to obtain quantitative *in vitro* correlative and *in vivo* data on possible mechanisms of the paradoxical actions of ethanol and thus provide leads for novel therapeutic approaches.

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