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Optical spectroscopy and prevention of deleterious cerebral vascular effects of ethanol by magnesium ions

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Abstract

Previously, it has been suggested that acute ethanol (alcohol) administration can result in concentration-dependent vasoconstriction and decreased cerebral blood flow. Here, we present in vivo results using rapid (240 nm/min) optical backscatter measurements, with an intact cranial preparation in the rat, indicating that acute infusion of ethanol directly into the rat brain rapidly produces dose-dependent vasoconstriction of the cerebral microcirculation associated with a pronounced reduction in tissue blood content, pronounced rises in deoxyhemoglobin, significantly increased levels of reduced cytochrome oxidase and microvascular damage as the dose increases. Furthermore, we present in vivo experiments demonstrating the capability of magnesium ions (Mg^{2+}) to attenuate and prevent these deleterious responses. Optical backscatter spectra (500–800 nm) were obtained by directing a single sending and receiving fiber to a portion of the left parietal cranium (in anesthetized rats), shaved to a translucent appearance to facilitate optical penetration. In the absence of added Mg^{2+}, infusion of a 10% solution of ethanol at 0.34 ml/min (~26.8 mg/min) produced prompt vasoconstriction as evidenced by a greater than 90% loss of oxyhemoglobin from the field-of-view and increases in levels of reduced cytochrome oxidase to between 50% and >90%. These effects were partially, to nearly completely, attenuated by the addition of $MgCl_2$ to the infusate containing added ethanol. Of special interest was the observation that attenuation of the vasoconstrictive effect of ethanol by Mg^{2+} persisted despite a subsequent ethanol challenge without added Mg^{2+}. The results obtained demonstrate that, depending on dose, ethanol can produce prompt and severe vasoconstriction of the intact cerebral microcirculation and that infusion of moderate doses of Mg^{2+} can largely attenuate and prevent this response. We conclude that appreciable, graded changes in cerebral cytochrome oxidase aa_3, blood volume and the state of hemoglobin occur at minimal tissue levels of ethanol which can be modulated by Mg^{2+}. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Magnesium; Alcohol; Cerebral microcirculation; Cytochrome oxidase; Hemoglobin; Stroke; Optical spectroscopy

1. Introduction

Clinical studies have shown that chronic abuse of alcohol can produce a variety of effects which may be related to a reduction in cerebral blood flow (Altura and Altura, 1984a; Berglund, 1981; Porjesz and Begleiter, 1983). These include atrophy of cortical, subcortical and cerebral areas of the brain, associated with blackouts, functional neuronal deficits and psychoses. Clinically, it is known that alcohol abuse and "binge" drinking can result in hemorrhagic strokes and cerebral infarctions (Altura and Altura, 1984a; Camargo, 1989; Donahue et al., 1986; Hillbom and Kaste, 1978). Previous studies from our laboratory, using image-splitting in vivo television microscopy, have shown that acute infusion of ethanol, produced graded concentration-dependent spasms of cortical arteries and venules in the intact rat brain causing, at high concentrations (~200–300 mg/dl), rupture of the venular microvessels (Altura et al., 1983; Altura and Altura, 1994) resembling stroke-like events. In addition, it has been shown in intact, unopened rat brains, using ^{31}P-nuclear magnetic resonance (NMR) spectroscopy, that systemic administration of ethanol can produce concentration-de-

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pendent brain ischemia, preceded by rapid falls in brain intracellular free Mg^{2+} ($[Mg^{2+}]_i$) (Altura et al., 1991; Altura and Altura, 1994). Increasing doses of ethanol precipitate hemorrhagic strokes in these rat models preceded by falls in brain phosphocreatine and ATP and rises in intracellular inorganic phosphate levels (Altura et al., 1991; Altura and Altura, 1994). Several additional studies on the intact rat brain, using in situ ^{31}P-NMR spectroscopy, suggest that administration of Mg^{2+} can prevent these hemorrhagic strokes (Altura and Altura, 1994; Altura et al., 1995a,b). Whether or not these effects of Mg^{2+} result in diminution or a complete loss of the mitochondrial ischemic events in the brain caused by alcohol is not known.

There is a growing body of both clinical and experimental literature to suggest that central nervous system (CNS) injury usually results in early and pronounced alterations in blood and brain levels of Mg^{2+} (Altura and Altura, 1982, 1984a,b; Bareyre et al., 1999; Cernak et al., 2000; Heath and Vink, 1998a; Helpern et al., 1993; Memon et al., 1995; Vink et al., 1987). Mg^{2+} deficiency prior to induction of experimental brain injury with ethanol and percussion injury is associated with higher mortality and worsened neurological outcomes (Altura and Altura, 1994; Altura et al., 1995a,b, 1998a,b; McIntosh et al., 1988).

Optical near-infrared spectroscopy has recently been used as a noninvasive technique to determine and measure cerebral oxygen availability in the intact brain (Hampson et al., 1990; Villringer and Chance, 1997). In the study herein, using rapid optical backscatter measurements, and visible and near-infrared optical spectroscopy, we have tested the hypothesis that administration of magnesium chloride will largely block ischemic effects of ethanol in the brain as determined by noninvasive measurements of mitochondrial cytochrome oxidase aa_3, deoxyhemoglobin and microvascular damage.

2. Materials and methods

2.1. Animal model, optical spectroscopy and protocol

A description of the methods used for the surgical preparation of the animals has been described previously (Lassoff et al., 1982). Briefly, male Wistar rats (175–230 g) were anesthetized with sodium pentobarbital (35–45 mg/kg, i.m., Nembutal) and cannulae were placed into a branch of the internal carotid artery (PE10) and a femoral vein (PE20). To improve optical penetration, the skull was exposed and shaved to a translucent appearance (Lassoff et al., 1982). The animal was placed in a prone position and the head stabilized by use of a three-point stereotaxic positioner. The overlying tissue was resected and the calvarium thinned by careful scraping with a scalpel until the outline of the cerebral vessels was evident. Repeat infusions of small doses of pentobarbital (3–5 mg) were made as needed to maintain a light plane of surgical anesthesia.

2.2. Optical measurements

Fiber optic bundles were used to intercept light entering the sample and reference cells of a Perkin-Elmer Lambda 5 Spectrophotometer. The bundle from the sample port was directed to a 20× Newport microscopic objective lens that focused the light onto the end of a 1-mm diameter single glass fiber. The fiber was used to illuminate the tissue. A second, receiving fiber, positioned 2.5–3.0 mm from the illuminating fiber, served to capture the backscattered signals. The fiber optic bundle from the reference port and the receiving fiber were directed to a homemade "black-box" containing a Hamamatsu model 1463 end-on photomultiplier tube. The intensity of the reference signal could be adjusted by varying the aperture of an adjustable iris.

The system was calibrated against the 656.1 nm emission line from a deuterium lamp. On each day of use, the intensity of the optical signals from the sample and reference fibers was balanced by performing a background correction against a barium sulfate planchet. Once calibrated, the fibers were oriented normally to the surface and placed in light contact with the thinned calvarium. A drop of microscope oil was placed at the point of contact to improve optical coupling and stabilize the signal. Optical measurements were made by scanning at 240 nm/min between 800 and 500 nm in steps of 1 nm resolution with the instrument set in the transmittance mode. Results presented are displayed in the absorbance mode.

2.3. Interpretation of optical spectra

2.3.1. Relative blood content

Variations in the relative blood content in the viewing field were estimated by comparing the intensity of the signal about the isobestic point in the range of 586–591 nm from control spectra (no infusion) to spectra obtained during infusion of Ringers solution at high flow rates (0.8 ml/min). Under these conditions, global blanching of the brain was apparent, with no evidence of the hemoglobin signal being present and only the background-reduced cytochrome spectrum could be seen. At wavelengths below 600 nm, the amplitude of the latter was reduced by $\sim$ 40% when compared with control spectra. The wavelength internal for the apparent "isobestic point" was derived by examining multiple sets of control spectra and spectra from KCl-arrested animals ($n = 6$). This interval is not indicative of instability in the measurements, but rather likely, results from differences among the preparations in coupling efficiencies and thickness of the overlying cranium. This is supported by two lines of evidence: (1) replicate measurements in control animals were reproducible to within 1%, and (2) the observed signal-to-noise level is typically greater than 50:1.

2.3.2. Level of deoxyhemoglobin

An estimate of the level of deoxyhemoglobin in the animal spectra was made by comparing differences in signal

intensity at 576 and 587 nm to measurements performed using the same set-up on mixtures containing whole rat blood with added 5% v/v microscopic latex beads (2.02 μm, 10% solids, Seragen Diagnostics) that had been equilibrated to different O_2 tensions ($n=6$). The latter was accomplished by varying a mixture of gases containing 95% O_2/5% CO_2 and 95% N_2/5% CO_2. The corresponding oxygen saturation of the mixtures (without added latex beads) was independently determined using a Radiometer OSM3 oximeter operated in the animal mode for a rat. The mean value of hemoglobin O_2 saturation calculated from 15 animal control spectra by this method was 91 ± 4%, which is similar to that found in vivo.

2.3.3. Levels of reduced cytochrome oxidase

Levels of reduced cytochrome oxidase were estimated by comparing the difference in signal intensity at 605 and 620 nm The differences seen in control spectra and KC1-arrested animals were defined as fully oxidized and fully reduced, respectively. Intermediate values were calculated by linear interpolation.

Difference spectra shown were computed by subtracting the data obtained, at the indicated infusion rate, with Ringers solution alone (control) from data obtained, at the same infusion rate, in the presence of added ethanol and ethanol plus added $MgCl_2$.

A total of 38 male Wistar rats were used in these studies including six animals used in the Mg^{2+} coadministration experiments. Continuous infusion was performed using a Harvard infusion pump set at flow settings of 0.07, 0.14 and 0.34 ml/min. The infusate was Ringers solution with and without added 10% ethanol. In some experiments, the latter was supplemented with added magnesium chloride at concentrations between 5 and 20 mM. Control studies involved infusing Ringers solution at the above flow rates and optical scans were started within 30 s after initiating the infusion. Following this, solutions containing 10% ethanol were infused at the different flow rates using a similar protocol. Subsequently, a solution containing added magnesium chloride (5–20 mM) and 10% ethanol was infused at the indicated flow rates. In four of six preparations, the initial concentration of magnesium chloride tested was 5 and 10 mM for the remaining two. For the former, in two preparations, minimal or only moderate attenuation of the ethanol-induced vasoconstrictive response was observed. In these cases, following a 10–20-min period of no infusion, a subsequent ethanol challenge at the same flow rate (0.34 ml/min) was given but with a higher concentration of added magnesium ions.

2.4. Statistics

Where appropriate, means ± S.E.M. were calculated and compared using paired and unpaired *t*-tests as well as analysis of variance (ANOVA) with Scheffe's contrast test. A *P*-value less than 0.05 was considered significant.

3. Results

Results revealing the sensitivity of the optical spectra, obtained from cerebral tissue, to a constant infusion of 10% ethanol at the indicated flow rates, with and without added $MgCl_2$, are shown in Figs. 1–4. In each case, the sequence of the infusion protocol involved Ringers alone, Ringers plus 10% ethanol, and Ringers plus added ethanol plus $MgCl_2$. Because of differences in the infusion protocol and range of responses observed, a total of four representative sets of data is given.

Results in Fig. 1A (primary spectra) and Fig. 1B (difference spectra) show that infusion of a 10% ethanol solution produces a dose-dependent vasoconstrictive response resulting in, at the highest flow rate (0.34 ml/min), near complete exclusion of the hemoglobin signal from the field-of-view of the receiving fiber. The resultant spectra reveal the background tissue cytochromes in their reduced state and

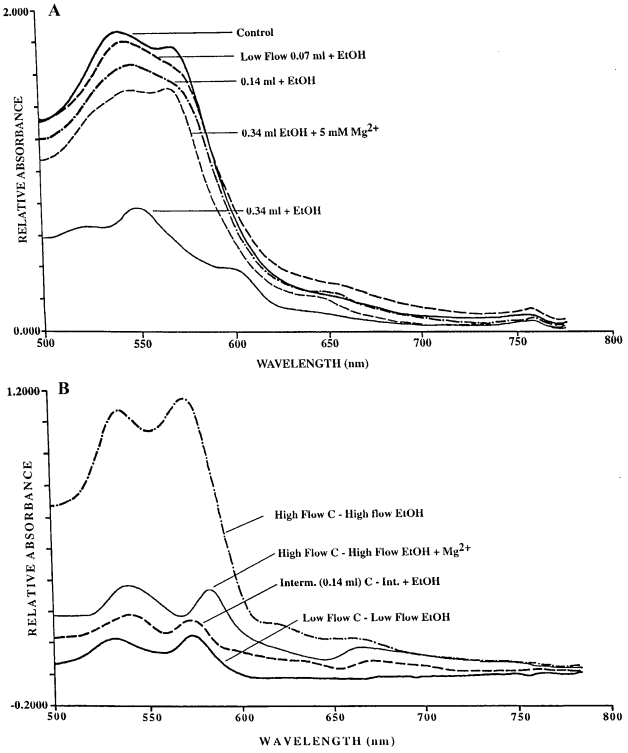

Fig. 1. Optical backscatter measurements on rat brain. Panel A, primary spectra. Infusion protocol was as follows: (, thick line), control, infusion of Ringers solution at 0.07 ml/min; thick line, 10% ethanol in Ringers at 0.07 ml/min; (, thick line), 10% ethanol in Ringers at 0.14 ml/min; (, thin line), 10% ethanol in Ringers at 0.34 ml/min; (, thin line), 10% ethanol, 5 mM $MgCl_2$ in Ringers at 0.34 ml/min. See Materials and methods for description of surgical procedures and optical measurements. Panel B, difference spectra. Difference spectra were obtained by subtracting the infusion rate match controls of Ringers solution from data obtained, at the same infusion rate, with Ringers and added ethanol, and added ethanol plus $MgCl_2$ (, thick line), 0.07 ml/min Ringers minus 0.07 ml/min 10% ethanol; (, thick line), 0.14 ml/min Ringers minus 0.14 ml/min 10% ethanol; (, thick line), 0.34 ml/min Ringers minus 0.34 ml/min 10% ethanol; (, thin line) 0.34 ml/min Ringers minus 0.34 ml/min 10% ethanol, 5 mM $MgCl_2$.

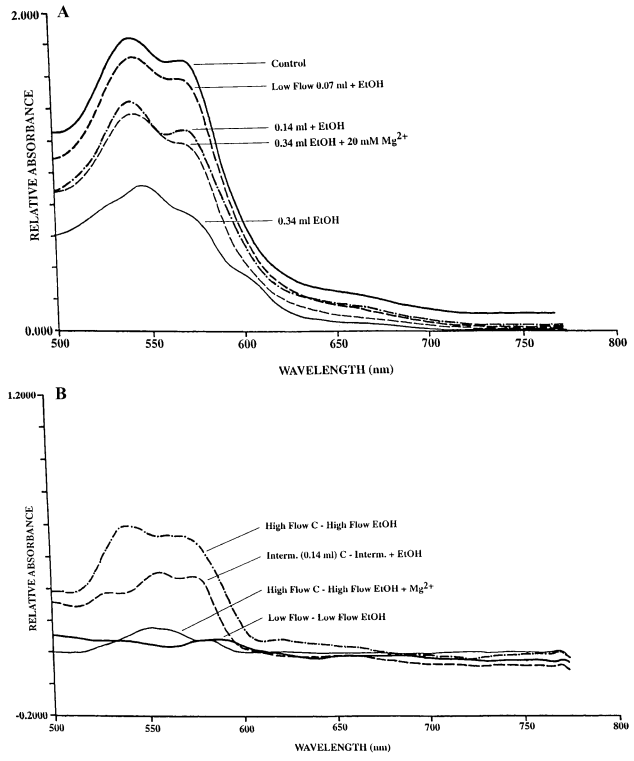

Fig. 2. Optical backscatter spectra of rat brain. Panel A, primary spectra; Panel B, difference spectra. Labeling of spectra is the same as listed in the legend of Fig. 1 with the exception that 20 mM $MgCl_2$ was included in the infusate.

are essentially indistinguishable from that observed 60 min following death of the animal or by infusing Ringers solution at flow rates high enough to exclude blood from the brain (>1.36 ml/min). The peaks at approximately 550 and 605 nm are consistent with the known absorption maximum of reduced cytochrome $c+c_1$, and aa_3, respectively (Tamura et al., 1978).

At the highest flow rates, the ethanol-induced vasoconstrictive response is even observable by the unaided eye in room light, and is seen as a global blanching of the tissue. Significantly, when 5 mM $MgCl_2$ was included in the infusate containing 10% ethanol, the hemoglobin signal recovered to approximately 75% of the control level. A similar finding was seen in two other preparations (results not shown). While estimates of the level of deoxyhemoglobin in this spectra are difficult to quantify due to the change in the signal intensity at the reported isobestic point (approximately 587 nm), the observation that the absorption maximal at approximately 545 and 576 nm have nearly equivalent amplitudes, and the lack of any distinctive peak at 560 nm would suggest it is low. This finding, together with the observed increase in the total hemoglobin signal, clearly indicates that coadministration of $MgCl_2$ at a dose of 1.7 μmol/min significantly attenuates the profound vasoconstrictive effect caused by infusion of 10% ethanol.

Examination of spectra shown in Fig. 2A (primary spectra) and Fig. 2B (difference spectra) reveals a qualitatively similar response to that seen in Fig. 1. Quantitative differences although are apparent, particularly with respect to the response of coadministered $MgCl_2$. At the highest infusion rate, pronounced vasoconstriction by ethanol is evident. However, the occurrence of a shoulder at approximately 576 nm (Panel A) indicates the presence of hemoglobin at levels higher than observed in Fig. 1. A subsequent coadministration of $MgCl_2$ at concentrations of 5 or 10 mM, at the same infusion rate in this preparation, failed, however, to attenuate the ethanol-induced vasoconstriction to any significant extent (results not shown). At concentrations of 20 mM $MgCl_2$, though near complete recovery was observed as seen in the difference spectrum in Panel B. Note the finding that the intensity of the primary spectra shown in Panel A, in the presence of added $MgCl_2$ although less that the 0.07 ml/min Ringers control is not inconsistent with this observation. The intensity of the match flow control (0.3 ml/min Ringers) performed immediately following this infusion, was also reduced, suggesting that some hemodilution had occurred as a result of the multiple infusions. A similar resistance to attenuation of the ethanol-induced vasoconstriction with 5 mM $MgCl_2$ was observed in one other preparation. In this case, significant attenuation of the ethanol effect (approximately 70%) occurred with infusion

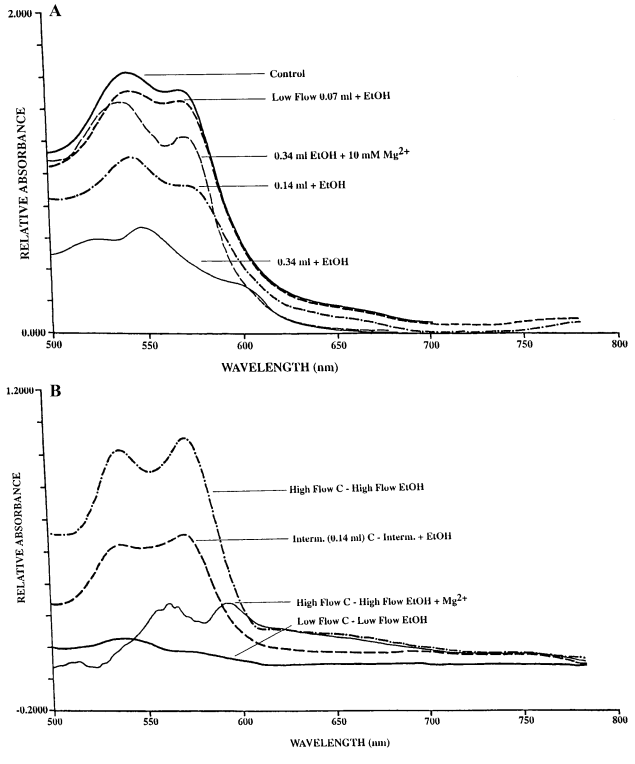

Fig. 3. Optical backscatter spectra of rat brain. Panel A, primary spectra; Panel B, difference spectra. Labeling of spectra is the same as listed in the legend of Fig. 1 with the exception that 10 mM $MgCl_2$ was included in the infusate.

of 10 mM $MgCl_2$. Also observed in Fig. 2 is the apparent greater sensitivity to the intermediate rate of ethanol infusion (0.14 ml/min) than that seen in Fig. 1.

Spectra shown in Fig. 3A (primary spectra) and Fig. 3B (difference spectra) also reveal results qualitatively similar to those in Fig. 1 but with quantitative features different from those observed in Fig. 2. At the highest infusion rate, the vasoconstrictive effect of ethanol was greater than that seen in Fig. 2 and similar to the effect seen in Fig. 1. However, at the intermediate infusion rate, the opposite trend was seen; the effect was similar to that seen in Fig. 2 and greater than the effect seen in Fig. 1. A subsequent coadministration of 10 mM $MgCl_2$, produced a significant (approximately 80%) attenuation of the ethanol-induced vasoconstriction.

The response of the cerebral microvasculature to 10% ethanol infusion, coadministration of ethanol with 10 mM $MgCl_2$, and to a subsequent ethanol challenge in the absence of added $MgCl_2$ is seen in Fig. 4A (primary spectra) and Fig. 4B (difference spectra). At the highest rate of infusion, the magnitude of the ethanol-induced vasoconstriction in this preparation is considerably less than seen in the previous figures. While these data are qualitatively similar to results shown in the previous figures, it is apparent that

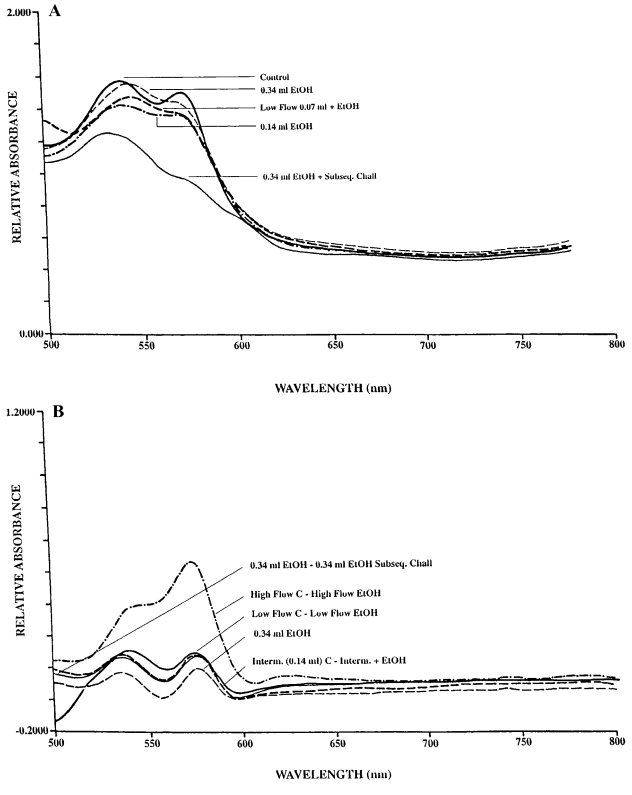

Fig. 4. Optical backscatter spectra of rat brain. Panel A, primary spectra; Panel B, difference spectra. Labeling of spectra is the same as listed in the Legend of Fig. 1 with the following exceptions: Panel A, (, thin line), 10% ethanol in Ringers at 0.34 ml/min, subsequent challange; Panel B, (, thin line), 0.34 ml/min Ringers minus 0.34 ml/min 10% ethanol, subsequent challenge.

Table 1
Cerebral vascular effects of ethanol in the intact rat brain: attenuation by $MgCl_2$

Ethanol dose (mg/min)	*n*	$MgCl_2$ (μmol/min)	Infusion rate (ml/min)	Total hemoglobin (%)	Reduced cytochrome aa_3 (%)
0	6	0	0.07–0.34	95–100	<10
5.5	6	0	0.07	92.5 ± 4.3[a] (85–>95)[b]	<10
11.0	6	0	0.14	44.8 ± 2.4[c] (25–60)	<10–25
26.8	6	0	0.34	20.2 ± 1.6 (0–<50)	50–>90[c]
26.8	6	1.7–6.8[d]	0.34	86.8 ± 6.8 (70–>90)	<10
26.8[e]	2	0	0.34	>90	<10

[a] Values are means ± S.E.M.
[b] Range of values recorded.
[c] Significantly different from 0-ethanol ($P<0.01$).
[d] See text for description.
[e] Subsequent challenge.

the quantitative response to ethanol infusion varies among the different preparations. As shown in Fig. 4A, coadministration of 10 mM $MgCl_2$ to the 10% ethanol infusate nearly completely attenuated the ethanol effect. It should be noted that the primary spectrum for this infusion protocol, shown in Fig. 4A, overlaps nearly completely with the spectrum obtained for infusion of 10% ethanol at 0.07 ml/min. Of particular interest in this set of experiments, however, was the observation that a subsequent ethanol challenge in the absence of added $MgCl_2$, performed 10 min following the previous infusion, failed to produce the expected vasoconstrictive response suggesting either a threshold effect or a hysteresis in the vascular response.

A summary of the above results is given in Table 1.

4. Discussion

Currently, the available techniques used for diagnostic brain imaging can be classified into structural and functional imaging methods. Structural imaging of the brain is used to acquire anatomical information (e.g., X-ray-computed tomography [CT], magnetic resonance imaging [MRI], and ultrasound imaging) while the goal of functional imaging of the brain is to acquire information on the physiological state of cerebral tissue (e.g., blood flow, oxygen consumption, metabolic activity, neuronal activity, etc.). These methods include functional MRI (fMRI), electroencephalography (EEG), magnetoencephalography (MEG), position emission tomography (PET) and single photon emission computed tomography (SPECT). Near-infrared spectroscopy (NIRS) was designed to measure concentration changes in hemoglobin and cytochromes in the brain noninvasively (Hoshi and Tamura, 1993; Jöbsis, 1997). NIRS, although primarily utilized to assess brain tissue oxygenation, has also demonstrated considerable potential

for neuroimaging (e.g., functional NIRS) (Hirth et al., 1996; Hoshi and Tamura, 1993; Kato et al., 1993). Recently, noninvasive approaches have utilized near-infrared light to interrogate the humar cortex through the intact scalp and skull (Villringer and Chance, 1997). It is now thus possible, as utilized herein to employ visible and near-infrared light to illuminate the brain.

The present results, using optical reflectance spectroscopy, confirm and extend previous work of others that have utilized only invasive techniques (e.g., isotopes, in vivo microcirculatory studies) and ^{31}P-NMR spectroscopy to assess dynamics of cerebral blood flow changes in the intact brain in response to administration or ingestion of various doses of ethanol (Altura and Altura, 1994; Altura et al., 1983, 1991; Berglund, 1981; Friedman et al., 1984; Goldman et al., 1973; Hadji-Dimo et al., 1968). The increased, observed incidence of strokes seen in human subjects after alcohol abuse and binge drinking, and the controversy concerning the mechanism(s) of alcohol-induced strokes, makes the noninvasive approach taken herein of special importance. Although the optical approach taken herein does not allow one an examination of discrete, microscopic localized areas of the brain microvasculature (i.e., arterioles, venules, or capillaries per se), it can discern noninvasively tissue oxygenation, blood volume, the mitochondrial state, and degree of tissue ischemia in a closed cranium, thus allowing rapid, repeat or continuous assessment of blood flow distribution prior to, during, or postischemic (or stroke-like) syndromes.

Since the accepted distribution ratio of ethanol in blood is 1.21 (Wallgren, 1970), the expected brain blood ethanol levels for the present study, would be 9–51 mg/dl, depending upon dose-infusion rate. The fact that such low concentrations of ethanol in the brain blood produce concentration-dependent vasoconstriction, which disappears (over time) and is reversible, makes the present findings more than of just passing experimental interest. These approximate brain blood concentrations of ethanol are clearly below the present legal limits for driving a motor vehicle in the 50 states of the U.S.A., and as such, if extrapolated to human subjects, suggest that various areas in the brain would be expected to undergo ischemic events with less than the equivalent of one standard alcohol drink.

Because of the great dependence of cerebral function on oxidative metabolism, occurrence of such ischemic events could significantly interfere with the oxidative capacity of the cerebral tissues leading to desaturation of oxyhemoglobin in the capillary bed, as we have found in this study, and a resultant reduction of mitochondrial function, as indicated in this study by the precipitous concentration-dependent rise in reduced cytochrome oxidase. With respect to the latter, the results in the present study suggest that mitochondrial oxidative function would be severely compromised due to the high levels (50–90%) of reduced cytochrome oxidase. In this context, our results using direct visual inspection revealed that the observed alcohol-induced ischemic responses occurred over a very broad brain area indicated by a global blanching of the tissue. Upon cessation of the ethanol infusion, often a pronounced hyperemic overshoot is observed, consistent with previous studies demonstrating such a response following a hypoxic insult (Altura et al., 1983).

Since more than 90% of the O_2 consumed by animals and humans involves cytochrome aa_3 or complex IV (cytochrome-*c*-O_2 oxidoreductase) (Capaldi, 1990), the present study suggests that alcohol induced decreases in brain O_2 content (indicated by >75–90% increases in deoxygenated hemoglobin) reduces the cerebral cytochrome aa_3 redox state. Others have reported previously that when oxygen saturation of arterial blood reaches 88% in humans, significant reduction of cytochrome aa_3 is observed (Hampson et al., 1990). Most importantly, our present study also demonstrates that coadministration of Mg^{2+} with ethanol results in a reversibility of the dramatic increase in reduced cytochrome oxidase.

An important role for Mg^{2+} in the pathophysiology of brain injury has been suggested by numerous studies which indicate that brain injury, including that induced by alcohol, is accompanied early on by decreases in brain total Mg^{2+} and $[Mg^{2+}]_i$ (Altura and Altura, 1982, 1984a, 1994; Altura et al., 1991, 1995a; Helpern et al., 1993; McIntosh et al., 1988; Vink et al., 1987) concomitant with marked depression in blood free Mg^{2+} levels (Altura and Altura, 1994; Altura et al., 1991, 1994, 1995a,b, 1997b; Bareyre et al., 1999; Heath and Vink, 1998a; Memon et al., 1995); ionized levels of Mg^{2+} are clearly affected more than total Mg. Ingestion of alcoholic beverages prior to brain trauma clearly intensifies the depression in circulating levels of Mg^{2+} in human subjects (Altura et al., 1995b). Dietary deficiency in Mg intake for short periods of time is associated with significantly higher mortality to administration of alcohol and higher stroke mortality (Altura et al., 1998a). Such dietary deficiency in Mg intake has been demonstrated to rapidly (over a few days) lower, significantly, brain $[Mg^{2+}]_i$ levels (Altura et al., 1997a). Prior to or following brain injury, Mg^{2+} administration has been shown to reduce decreases in brain $[Mg^{2+}]_i$, reduce regional cerebral edema, reduce decreases in blood levels of ionized Mg^{2+} and $[Ca^{2+}]_i$, reduce neuromotor deficits, and reduce memory loss (Altura and Altura, 1994; Altura et al., 1995b; Bareyre et al., 2000; Feldman et al., 1996; Heath and Vink, 1998b; McIntosh et al., 1989). In addition, Mg^{2+} has been shown to be neuroprotective in hippocampal slice preparations subjected to excitotoxic or anoxic insults (Clark and Rothman, 1987; Kass et al., 1988) as well as lessen hippocampal neuronal degeneration caused by excitatory amino acids or global ischemia (Fischer et al., 1993; Tsuda et al., 1991; Wolf et al., 1991). Our present data, as well as data acquired in alcohol-induced stroke (Altura and Altura, 1994; Altura et al., 1991, 1995a), are consistent with these findings. The present findings indicating that Mg^{2+} infusion prevents or ameliorates ethanol-induced mitochondrial dys-

function (as observed by precipitous rises in reduced cytochrome oxidase), are consistent with findings showing that decreased extracellular Mg^{2+} results in increased cortical neuronal cell death due to oxidative injury (Regan et al., 1998). Since ethanol administration to rats results initially and rapidly (less than 2 min) in marked loss of brain $[Mg^{2+}]_i$ (Altura and Altura, 1994; Altura et al., 1991, 1995a), Mg^{2+} infusion could be expected to ameliorate alcohol-induced brain injury.

We have shown previously that decreased extracellular Mg^{2+} results in significantly increased intracellular free Ca^{2+} ($[Ca^{2+}]_i$) in dissociated hippocampal neurons (Zhang et al., 1996), cerebral vascular muscle cells (Altura and Altura, 1982, 1984b, 1994), and type-2 astrocytes (Altura and Altura, 1994; Altura et al., 1983). It is, thus, likely that the alcohol-induced loss of brain $[Mg^{2+}]_i$ by inducing Ca^{2+}-dependent cerebral microvascular constriction (Altura and Altura, 1994), followed by a proinflammatory response (Altura and Gebrewold, 1996, 1998), induces vascular smooth muscle, endothelial and neuronal cell damage (Altura and Altura, 1994; Altura et al., 1983, 1991; Altura and Gebrewold, 1996, 1998; Yang et al., 2001; Zhang et al., 1993). An early biochemical marker of these events, from the work described herein, appears to be concentration-dependent increases in reduced cytochrome oxidase.

Since mitochondria may regulate cell death (i.e., apoptosis) (Green, 2000; Lockshin and Zakeri, 2001), and an early marker of this event is a release of mitochondrial cytochrome *c* into the cytoplasm (Green, 2000), it is possible that the early and rapid appearance of increased brain reduced cytochrome oxidase levels, which are seen in the present study, could be indicative of such early apoptotic events with increasing doses of ethanol. In this vein, it has recently been demonstrated that ethanol induces apoptosis in a variety of tissues including the brain (Zhang et al., 1998), and the alcohol-induced apoptosis can be activated by the cytochrome *c* pathway (Zhou et al., 2001). If this hypothesis is borne out, then Mg^{2+} may turn out to be an inhibitor of some apoptotic events in brain injury. In this context, it is important to point out that traumatic brain injury that leads to cell death can be markedly attenuated by Mg^{2+} treatment and exacerbated by Mg^{2+}-deficiency (Altura and Altura, 1994; Altura et al., 1995a, 1998a,b; Bareyre et al., 2000; Feldman et al., 1996; Fischer et al., 1993; Heath and Vink, 1998b; McIntosh et al., 1988, 1989; Tsuda et al., 1991; Wolf et al., 1991). Lastly, the present results suggest that it may be possible to rapidly monitor noninvasively, the therapeutic benefits and actions of Mg^{2+} and other ameliorative agents in the intact human brain by use of optical reflectance spectroscopy.

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