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Optical Spectroscopy and Prevention of Deleterious Brain- damaging Cerebral Vascular Effects of Cocaine by Magnesium Ions: Effects on Brain Mitochondrial Oxidase, Deoxyhemoglobin, Ceramide and Sphingomyelin Levels and Their Potential Application to Human Substance Abuse

Research Article

Altura BM[1-7*], Gebrewold A[1], Carella A[1], Barbour RL[8], Wu F[1], Altura BT[1, 3-7]

[1] Department of Physiology and Pharmacology, The State University of New York Downstate Medical Center, Brooklyn, New York, USA.
[2] Department of Medicine, The State University of New York Downstate Medical Center, Brooklyn, New York, USA.
[3] The Center for Cardiovascular and Muscle Research, The State University of New York Downstate Medical Center, Brooklyn, New York, USA.
[4] The School for Graduate Molecular and Cellular Science, The State University of New York Downstate Medical Center, Brooklyn, New York, USA.
[5] Bio-Defense Systems, Inc, Rockville Centre, New York, USA.
[6] Orient Biomedica, Estero, Florida, USA.
[7] Magnesium for Health Foundation, Patterson, California, USA.
[8] Department of Pathology, State University of New York, Brooklyn, New York, USA.

Abstract

Previously, four of us suggested that acute cocaine HCl administration can result in concentration-dependent vasoconstriction, vasospasm and decreased cerebral blood flow. Here, we present new *in vivo* results using rapid (240 nm/min) optical backscatter measurements, with an intact cranial preparation in the rat, indicating that acute infusion of cocaine HCl directly (via branch of internal carotid) into the rat brain rapidly produces dose-dependent vasoconstriction of the cerebral microcirculation associated with a pronounced reduction in tissue blood content, pronounced elevation in deoxyhemoglobin, significantly increased levels of reduced cytochrome oxidase aa_3 and microvascular damage as the dose of cocaine was increased. Furthermore, we present *in vivo* experiments demonstrating the capability of magnesium ions (Mg^{2+}) to attenuate and prevent these deleterious responses of cocaine HCl. 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 solution of cocaine HCl at 0.34 ml/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 infusion containing added cocaine HCl. Of particular interest was the observation that attenuation of the vasoconstrictive effects of cocaine by Mg persisted despite a subsequent cocaine challenge without added Mg^{2+}. In other experiments, based on previous studies, we noted rapid increases in production and cellular release of ceramides concomitant with reductions in brain sphingomyelin in response to cocaine administration which were either attenuated or inhibited by prior administration of either Mg^{2+} or a blocker of ceramide synthesis, namely, myriocin. Our new results indicate that, depending upon dose, cocaine HCl can produce prompt and severe vasoconstriction and that infusion of Mg^{2+} can largely attenuate and prevent this response. In addition, we demonstrate that infusion of cocaine directly into the brain results in rapid synthesis and release of ceramides which can be attenuated/inhibited by pretreatment of animals with either Mg^{2+} or myriocin.

Introduction

Clinical and experimental studies have now, unequivocally, established that ingestion, intravenously administered, or snorting of cocaine or abuse of cocaine can produce a variety of dangerous effects in different areas of the brain, including profound reductions in blood flows and strokes [1-7]. These actions include atrophy of cortical, subcortical, prefrontal cortical, hippocampal, medullary and cerebral areas of the brain associated with headaches, blackouts, functional neuronal deficits

***Corresponding Author**:
B. M. Altura,
Professor, Box 31, SUNY Downstate Medical Center, 450 Clarkson Ave., Brooklyn, NY 11203, USA.
Tel: 718-270-2194
E-mail: burton.altura@downstate.edu

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and pyschoses. Clinically, it is known that cocaine abuse can result in hemorrhagic strokes and cerebral infarctions [1-7]. The first report of cocaine-induced stroke was reported in 1977, more than 40 years ago by Brust and Richter [8].

Previous studies from our laboratories, using image-splitting *in vivo* television microscopy, have shown that acute infusion of cocaine HCl, produced graded concentration-dependent spasms of cerebral and medullary arterioles and arteries in the intact rat brain causing rupture of venular postcapillaries, thus resembling stroke-like events [9]. Moreover, examination of isolated canine, rat, monkey and baboon cerebral and basilar arteries contracted in a dose-dependent manner upon the addition of cocaine HCl in isolated organ baths maintained under physiologic conditions [10, 11]. In addition, we have shown in intact, unopened rat brains, using 31P-nuclear magnetic resonance (NMR) spectroscopy, that administration of cocaine HCl can produce concentration-dependent brain ischemia, preceded by rapid falls in brain intracellular free Mg^{2+} ($[Mg^{2+}]_i$) [12]. Increasing doses of cocaine HCl induced hemorrhagic strokes in these rat models preceded by falls in phosphocreatine and ATP and rises in intracellular phosphate levels [12]. Several additional studies on the intact rat brain, using in situ ^{31}P-NMR spectroscopy and direct in-vivo observations of the cerebral microcirculation suggest that administration of Mg^{2+} can prevent these hemorrhagic strokes [9, 12]. Whether or not these effects of Mg^{2+} result in diminution or a complete loss of mitochondrial ischemic events in the brain caused by cocaine 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+} [13-20]. Mg^{2+} deficiency prior to induction of experimental brain injury with cocaine and percussion injury is associated with higher mortality and worsened neurological outcomes [21].

Several years ago, optical near-infrared spectroscopy had been used as a noninvasive technique to determine and measure cerebral oxygen availability in the intact rat brain [22, 23]. In the study, herein, using rapid optical backscatter measurements, and visible near-infrared optical spectroscopy, we have tested the hypothesis that administration of magnesium chloride will largely block the ischemic effects of cocaine HCl in the brain as determined by noninvasive measurements of mitochondrial cytochrome oxidase aa_3, deoxyhemoglobin and microvascular damage.

Recently, our laboratories have provided evidence to indicate that low levels of $[Mg^{2+}]_0$ results in rapid synthesis and release of ceramides in canine and rat cerebral arterial smooth muscle cells and in all four chambers of rat heart cardiomyocytes [24-26]. We have reported that ceramides induce potent concentration-dependent contractions of canine and subhuman primate cerebral arteries [26-28]. In addition, using in situ high-resolution television-optics of the pial and medullary microvasculatures, we have found that many ceramides induce vasoconstriction-spasms of venules leading to adherence of leukocytes, macrophages and monocytes on the postcapillary venular walls [28]. In view of these new experiments, we hypothesized that if cocaine HCl resulted in rapid reductions in brain $[Mg^{2+}]_i$, like we have reported previously [12, 21], then infusion of cocaine into the brain should cause alterations in the levels of brain ceramides (i.e., rises) which might be inhibited or attenuated by a specific antagonist of ceramide synthesis like myriocin.

Materials and Methods

Animal model, optical spectroscopy and protocol

A description of the methods used for the surgical preparation of the animals has been described previously [29]. Briefly, male Wistar rats (175-230 g) were anesthetized with sodium pentobarbital (35-45 mg/kg, intramuscularly, Nembutal) and cannulae were placed into a branch of the internal carotid artery (PE10 tubing) and a femoral vein (PE20 tubing). To improve optical penetration, the skull was exposed and shaved, very carefully), to a translucent appearance [29]. The animal was then placed in a prone position and the head stabilized by use of a three-point stereotaxic positioner [29]. The underlying tissue was resected and the calvarium thinned by careful scaping with a scalpel until the outline of the cerebral vessels was evident [29]. Repeated infusions of small doses of pentobarbital (3-5 mg) were made as needed to maintain a light plane of surgical anesthesia [29].

Optical Measurements

Fiber optic bundles were used to intercept light entering the sample and reference cells of a Perkin-Elmer Lambda 5 spectrophotometer as described previously [30]. The bundle from the sample port was directed to a 20X Newport microscopic objective lens that focused the light onto the end of a 1-mm diameter single glass fiber [30]. 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 [30]. The fiber optic bundle from the reference port and the receiving fiber were directed to a homemade "black-box" containing a Hammamatsu model 463 end-on photomultiplier tube. The intensity of the reference signal could be adjusted by varying the aperture of an adjustable iris [30].

The system was calibrated against the 656.1 mm emission line from a deuterium lamp [30]. 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 couplingand stabilize the signal. Optical measurements were made by scanning at 240 mm/min between 800 and 500 nm in steps of 1 nm resolution with the instrument set in the transmittance mode [30]. Results were displayed in the absorbance mode.

Interpretation of optical spectra

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 [30]. At wavelengths below 600 nm, the amplitude of the latter was reduced by ~ 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=8). 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 underlying cranium [30]. This is supported by two lines of evidence: 1. Replicate measurements in control animals were reproducible; and 2. The observed signal-to-noise level is typically greater than 50:1.

Level of deoxyhemoglobin

An estimate of the level of deoxyhemoglobin in the animal spectra was madeby 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 beads (2.02 um, 10% solids, Seragen Diagnostics) that had been equilibrated to different O_2 tensions (n-6) [30]. 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*.

Levels of reduced cytochrome oxidase aa

Levels of reduced cytochrome oxidase were estimated by comparing the difference in signal intensity at 605 and 620 nm [30]. The differences seen in control spectra and KCl-arrested animals was defined as fully oxidized and fully reduced, respectively. Intermediate values were calculated by linear interpolation.

Difference spectra were computed by subtracting the data obtained, at the indicated infusion rate, in the presence of added cocaine HCl and cocaine HCl plus added $MgCl_2$, similar to methods we published previously [30].

A total of 78 male Wistar rats were used in these studies including animals used in the Mg^{2+} coadministration experiments. Continuous infusion was performed using a Harvard infusion pump at low settings of 0.07, 0.14 and 0.34 ml/min. The infusate was Ringers solution with and without added cocaine HCl. In some experiments, the latter was supplemented with added $MgCl_2$ 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 cocaine HCl were infused at the different flow rates using a similar protocol. Subsequently, a solution containing added $MgCl_2$ (5-20 mM) and cocaine HCl was infused at the indicated flow rates. In six of eight preparations, the initial concentration of $MgCl_2$ tested was 5 and 10 mM for the remaining two. For the former, in two preparations, minimal or only moderate attenuation of the cocaine HCl-induced vasoconstrictive response was observed. In these cases, following a10-20 min period of no infusion, a subsequent challenge at the same flow rate (i.e.,0.34 ml/min) was given but with a higher concentration of added $MgCl_2$.

Measurement of brain ceramides and sphingomyelin and use of myriocin or Mg

Using different groups of animals (six each), which were sacrificed by an overdose of pentobarbital sodium (100 mg/kg), several different experiments were performed: 1. infusion of cocaine HCl with or without $MgCl_2$; 2. Infusion of cocaine HCl with or without Ringers solution; and 3. Infusion of cocaine HCl after animals received either 10 mg/kg of myriocin or $MgCl_2$ given intravenously via tail vein injection.

Ceramides and sphingomyelin were measured in extirpated cerebral hemispheres after the foregoing groups of study protocols according to methods we previously published [26].

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 of less than 0.05 was considered significant.

Results

The data are summarized in Tables 1 and 2. Infusion of cocaine HCl showed that it produced a dose-dependent vasoconstrictive response resulting, 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 revealed that the background tissue cytochromes in their reduced state were essentially indistinguishable from that observed 60 min following death of the animal or by infusing Ringers solution at low flow rates high enough to exclude blood from the brain (>1.36 ml/min). The peaks of the spectra at approximately 550 and 605 nm were consistent with the known absorption maximum of reduced cytochrome c + c_1 and aa_3, respectively [31].

At the highest flow rates, the cocaine-induced vasoconstrictive response was even observable by the unaided eye in room light, and was seen as a global blanching of the brain tissue. Significantly, when 5 mM $MgCl_2$ was included in the infusate containing 0.5 % cocaine HCl, the hemoglobin signal recovered to approximately 85% of the control level. A similar finding was observed in two other animal preparations. While estimates of the level of deoxyhemoglobin in these spectra were difficult to quantify, due to the change in signal intensity, at the reported isobestic point (i.e., 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 quite low. This finding, together with the observed increase in the total hemoglobin signal, clearly indicates that the coadministration of $MgCl_2$ at a dose of 1.7 umol/min significantly attenuated the profound vasoconstrictive effect caused by infusion of cocaine HCl.

Coadministration of 10 mM $MgCl_2$ to the 0.5% cocaine HCl infusate nearly completely attenuated the cocaine vasoconstrictive effects. Although not shown, it should be noted, here, that the primary spectrum for this infusion protocol overlapped nearly

Table 1. Cerebral Vascular Effects of Cocaine HCL in the Intact Rat Brain: Attenuation by $MgCl_2$.

Cocaine (%)	n	$MgCl_2$ (umol/min)	Infusion Rate (ml/min)	Total Hemoglobin (%)	Reduced Cytochr aa_3
0	6	0	0.07-0.34	95-100	<10
0.01	6	0	0.07	91.5 +/-5.2	<10
0.25	6	0	0.14	42.3 +/- 2.12	<15-421
0.5	6	0	0.34	18.2 +/- 1.2 2	68-94
0.5	6	5	0.34	85.4 +/-4.4	14-36
0.5	6	1.7	0.14	64.6 +/-3.82	28-44
0.5	2	0	0.34	>90	<10

Values are means +/- S.E.M.
[1]Range of values
[2]Significantly different from all other hemoglobin mean values (P<0.01, ANOVA)

Table 2. Cocaine HCl Administration to Rats Results in Release of Ceramides and Reduction in Sphingomyelin (SM) in Cerebral Hemispheres.

Time, min	Ceramides (nmol/mg protein)	SM (nmol/mg protein)
0	39 +/- 3.5	380 +/- 28
30	68 +/- 7.2	198 +/- 24
45-75	78 +/- 7.4	216 +/- 18
45-75 with Mg	52 +/- 5.2	302 +/- 28
45-75 with Myriocin	44 +/- 3.2	358 +/- 32

Values are means +/- S.E. M. n=6 each
All paired experimental values (without either Mg or myriocin) are significantly different from controls at zero time (P<0.01).

completely with the spectrum obtained for infusion of 0.5% cocaine HCl at 0.07 ml/min. Of particular interest in this set of experiments, however, was the observation that a subsequent cocaine challenge in the absence of added $MgCl_2$, performed 10 min following the previous infusion, failed to produce the expected vasoconstrictive response, thus suggesting either tachyphylaxis, a threshold effect or a hysteresis in the vascular response.

Analysis of the concentrations of ceramides in the cerebral hemispheres 30 min after intravenously administered cocaine HCl, as predicted, indicated progressive rises in the synthesis of total ceramides (increasing with time elapsed), which was attenuated by $MgCl_2$, also as predicted, and completely inhibited by prior treatment with myriocin (see Table 2). Sphingomyelin levels were reduced with elapsed time and prevented from deficit reductions in the presence of either $MgCl_2$ or treatment with myriocin (Table 2).

Discussion

As of this writing, the available techniques used for diagnostic brain imaging can be classified into structural and functional imaging methods. Structural imaging of the brain is utilized 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 and other brain tissues (e.g., blood flows, oxygen consumption, metabolic activity, neuronal activity, etc.). These methods include functional MRIs (fMRIs), electroencephalography (EEG), magnetoencephalography (MEG), positron emission tomography (PET), and single photon emission computed tomography (SPECT). Near-infrared spectroscopy NIRS) was designed to measure concentration changes in hemoglobin and mitochondrial cytochromes in the brain, noninvasively [32,33]. NIRS, although primarily utilized to assess brain tissue oxygenation, has also demonstrated considerable potential for neuroimaging (e.g., functional NIRS) [32, 34-35]. Approximately 20 years ago, Villringer and Chance used noninvasive approaches employing near-infrared light to interrogate the human cortex through the intact scalp and skull [23]. It is now thus possible, as utilized herein, to employ visible light to illuminate the brain.

Our present results, using optical reflectance spectroscopy, confirm and extend our previous work and that of others that have utilized only invasive techniques (e.g., isotopes; *in vivo* microcirculatory studies) and 31P-NMR spectroscopy to assess dynamics of cerebral blood flow changes in the intact brain in response to administration or ingestion of various doses of cocaine [2, 3, 5, 9-12, 21]. The increased, observed incidence of strokes seen in human subjects after cocaine abuse, and the controversy concerning the mechanism (s) of cocaine-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, metarterioles,

precapillary sphincters, and venules or capillaries per se), it can discern noninvasively tissue oxygenation, blood volume, the mitochondrial state, and the degree of tissue ischemia in a closed cranium, thus allowing rapid, repeat or continuous assessment of blood flow distribution prior to, during or post-ischemic (or stroke-like) syndromes. In our opinion, the technology used, herein, is a major advancement, particularly in the substance abuse field.

Due to the great dependence of cerebral function on oxidative metabolism, occurrence of ischemic events in the brain could significantly interfere with the oxidative capacity of the brain cerebral tissues leading to the desaturation of oxyhemoglobin in the capillary bed, as found in the present study, and the resultant reduction of mitochondrial function as indicated by the precipitous concentration-dependent rise in reduced cytochrome oxidase. With respect to the latter, the results in the present study suggest that oxidative function would be severely compromised due to the high levels (i.e., 50-90%) of reduced cytochrome oxidase. These results remind us that Mg deficiency in rats yielded a very similar result, i.e., a precipitous rise in reduced cytochrome oxidase in cardiac myocytes, cerebral vascular smooth muscle cells, and cerebral-medullary tissue cells [26, 36-42, unpublished findings]. Whether the reduction in cerebral vascular smooth muscle cell, glial cell, and brain reduction in free $[Mg^{2+}]_I$ followed by membrane entry and release of intracellular Ca^{2+}, which we have reported on previously [41, 43-46], is in large measure responsible for the cocaine-induced reduction in mitochondrial metabolism, and is the major mechanism for mitochondrial dysfunction, remains to be determined. However, when the present findings are taken together with the cocaine-induced rises in brain ceramides , reported herein, we believe the latter is the most likely mechanism along with direct vasoconstrictive actions on cerebral arterial, arteriolar and precapillary vessels we have reported on previously [21, 24, 26-28].

Since more than 90% of the O_2 consumed by mammals and humans involves cytochrome aa_3 or complex IV (cytochrome-c-O_2 oxidoreductase) [47], the present study would suggest that cocaine-induced decreases in brain O_2 content (indicated by >75-90% increases in deoxygenated hemoglobin) must perforce reduce 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 [22]. Most importantly, the present study also demonstrates that coadministration of Mg^{2+} with cocaine HCL results in a reversibility of the dramatic increase in reduced cytochrome oxidase. The fact that administration of myriocin, by itself, markedly reduces the increase in reduced cytochrome aa_3, as well as resulting in an attenuation of the cocaine-induced increase in deoxyhemoglobin, clearly supports a role for synthesis and release of ceramides in the observed biochemical and biophysical cocaine-induced disturbances in mitochondrial functions. Our finding of a concomitant decrease in cerebral sphingomyelin (SM) (with time after cocaine) suggests that some of the rise in cerebral concentration of ceramides must be due to the breakdown of some SM. This probability is currently being explored in our labs.

Important roles for Mg^{2+} in the pathophysiology of brain injury and trauma have been suggested previously by numerous experimental and human studies which demonstrate that brain injury, including that induced by numerous substances of abuse (i.e., alcohol, PCP, marijuana-cannabis products, amphetamines, psilocybin, heroin, mescaline, etc.) is accompanied early by decreases in brain total and ionized Mg^{2+} [12, 13, 15, 21, 39, 48-60] concomitant with marked depression in blood free Mg^{2+} levels; in these cases, ionized levels of Mg^{2+} are clearly more affected than total Mg levels. Ingestion or injection of cocaine HCl prior to brain trauma clearly intensifies the depression in circulating levels of Mg^{2+} in human subjects [6]. We have found that dietary deficiency of Mg intake for short periods of time (i.e., 21 days) is associated with significantly higher mortality to subsequent administration of cocaine and higher stroke mortality [21]. In this context, our laboratory has reported that dietary deficiency in Mg intake can rapidly (even over a few days) lower brain free Mg levels but not necessarily brain total Mg levels [61].

Experimentally, using different forms of brain trauma, it has been demonstrated that administration of Mg will either prevent or reduce the losses in brain free Mg levels, reduce neuromotor deficits, and reduce memory loss in both anesthetized and freely moving animals [13, 17, 20, 21, 26, 62-65]. In addition, Mg^{2+} has been shown to be neuroprotective in hippocampal brain slice preparations subjected to neurotoxic or anoxic degeneration caused by excitatory amino acids or global ischemia [66, 67].

Our present data, as well as our data acquired in alcohol-, marijuana-cannabis-, amphetamines-, heroin-, fentanyl-, and psychedelic drug-induced strokes [9, 12, 17, 21, 51-60], are consistent with the latter findings. The present findings indicating that Mg^{2+} infusion prevents or ameliorates cocaine-induced mitochondrial dysfunction (as observed by precipitous rises in reduced cytochrome oxidase) are consistent with findings that decreased extracellular Mg^2 results in increased cortical cell death due to oxidative injury [68]. Since cocaine HCl administration to rats results in, initially and rapidly (less than 3 min), in marked losses of brain $[Mg^{2+}]_I$ [52], Mg^{2+} infusions could be expected to ameliorate cocaine-induced brain injury.

Previously, we have shown that decreased extracellular Mg^{2+} results in significantly increased intracellular free $[Ca^{2+}]_I$ in dissociated hippocampal neurons as well as in primary cultured type-2 rat astrocytes [17, 69, 70] and rat, canine and baboon primary cultured cerebral vascular smooth muscle cells [17]. It is, thus, likely that the cocaine-induced loss of brain $[Mg^{2+}]_I$ by inducing Ca^{2+}-dependent cerebral, cortical, and medullary vasoconstriction followed by a proinflammatory response (viz., increased levels of reactive oxygen species, cytokines such as IL-2 and TNF-alpha coupled to adherence of leukocytes, monocytes and macrophages to the brain venular postcapillary walls [21, 25, 26], clearly induces vascular smooth muscle, endothelial and neuronal cell damage [9, 12, 14, 15, 17, 25, 30, 38]. An early biomarker of these events, from the work described herein, appears to be concentration dependent increases in reduced cytochrome oxidase and rises in ceramides (Tables 1 and 2).

Since mitochondria may regulate cell death(i.e., apoptosis) [71, 72], and an early marker of this event is a release of mitochondrial cytochrome c into the cytoplasm [71, 72], 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 cocaine HCl. In this context, we have shown that cocaine HCl appears to induce apoptosis in a variety of tissues

(e.g., heart, peripheral vascular muscle, coronary arterial muscle cells, liver cells, among other) [21, 73, 74 unpublished findings], including the brain [73, unpublished findings]. Very recently, we have identified two additional pathways of programmed cell death in Mg-deficient cardiac, vascular muscle and brain tissues, viz., necroptosis and ferroptosis [for reviews, see 75, 76], both of which we have found in cocaine-treated animals [unpublished findings]. Both of these new cell death pathways activated by cocaine HCl were attenuated markedly by pretreatment of the animals with myriocin [unpublished findings]. If our hypothesis is borne-out, then coadministration of Mg^{2+} with blockers of ceramide synthesis, may turn out to be: 1. an inhibitor of several pathways involved in programmed cell death; and 2. useful in the treatment of cocaine-induced toxicity, tolerance, and brain damage, including strokes. Lastly, we believe our new findings could make it possible to rapidly monitor, noninvasively, the therapeutic benefits and actions of Mg^{2+} and ceramide antagonists in the intact human brain by use of optical reflectance spectroscopy.

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