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MAGNESIUM PROTECTS AGAINST COCAINE-INDUCED HEMORRHAGIC ^{31}P-NMR IN-VIVO STUDY

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1. ABSTRACT

In-vivo ^{31}P-nuclear magnetic resonance (NMR) studies were undertaken with anesthetized rats to determine: a. whether systemic administration of MgCl could protect animals against cocaine-induced hemorrhagic stroke, and b. whether a relationship exists between basal levels of brain intracellular free magnesium ions ($[Mg^{2+}]_i$), phosphometabolites, and stroke risk. Repeat ^{31}P-NMR spectra were obtained at various intervals of time (3-120 min, or up until death) after administration of cocaine (5 + 30 mg/kg). Ion selective electrodes were used to measure plasma Mg^{2+}, K^+, Na^+ and Ca^{2+}. Forty percent of animals died in the absence of Mg^{2+} infusion following high dosage of cocaine. Only 13% died with cocaine following Mg^{2+} infusion ($p < 0.005$). In the Mg^{2+}-protected animals, neither brain $[Mg^{2+}]_i$,intracellular pH (pHi), [phosphocreatine-PCr]/[ATP], nor brain [inorganic phosphate-Pi]/[ATP] fell when toxic and lethal doses of cocaine were given. Low basal brain $[Mg^{2+}]_i$ (275 ± 24 vs. 466 ± 35 µM, $p < 0.01$) and low basal brain [PCr] (3.36 ± 0.35 vs. 4.26 ± 0.24 mM, $p < 0.01$) were found to be associated with a 3-fold increased incidence of stroke. A positive correlation ($r = 0.31$, $p < 0.03$) between brain $[Mg^{2+}]_1$ and [PCr]/[ATP] was found. It is possible that both brain $[Mg^{2+}]_i$ and [PCr] may be useful as important predictors of susceptibility to cocaine-induced hemorrhagic stroke.

2. INTRODUCTION

Cocaine abuse results in an increased incidence of aneurysmal subarachnoid hemorrhages, intracerebral hemorrhages, brain edema and occlusion-type strokes in humans (for reviews see 1-3). Recently, it has been reported that cocaine administration to anesthetized rats can produce subarachnoid and intracerebral hemorrhagic strokes which result in rapid, significant deficits in whole brain intracellular free magnesium ions ($[Mg^{2+}]_i$, falls in intracellular pH (pHi), progressive loss of phosphocreatine (PCr) and elevation of inorganic phosphate (Pi) up until death (4). Other studies demonstrate that cocaine can produce direct vasospasm of cerebral blood vessels (5,6) concomitant with rapid loss of $[Mg^{2+}]_i$ (3) and cellular uptake/release of Ca^{2+} (8).

In vivo ^{31}P-NMR spectroscopic studies were undertaken to determine whether: 1. systemic administration of Mg^{2+} could protect animals against cocaine-induced hemorrhagic stroke; and 2. a relationship exists between basal levels of brain $[Mg^{2+}]_i$ and stroke risk. Since most cocaine abusers imbibe at least two or more doses of cocaine separated by intervals of time, in order to trigger the stroke (2,3,9), we developed a rat model to simulate the clinical experience.

3. METHODS

Male Wistar rats, weighing 135-180 g, were anesthetized lightly with pentobarbital sodium (Nembutal, 3 g/100 g, i.m.). After induction of anesthesia, each rat was placed in a General Electric Omega 400 WB spectrometer with a 9.4T vertical bore magnet utilizing double tuned ^{31}P/^{1}H RF coils (4). The animal was carefully accommodated in the NMR probe (with head pointing down) so that all of the brain was contained within the RF coil. In order to make certain that the brain was positioned properly, we also obtained proton images using S50

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Table 1: Effects of constant intravenous infusion of 10 µmol/min Magnesium chloride on brain $[Mg^{2+}]_i$, pH_i, and intracellular phosphometabolites as well as on plasma magnesium levels in normal animals

Group time-min (after $MgCl_2$)	$[Mg^{2+}]_i$ µM	pH_i	[PCr]/[ATP]	$[P_i]$/ATP]	$[Mg^{2+}]_o$ (mM)	Total Mg[a] (mM)
Controls	510 ± 22	7.27 ± 0.06	2.07 ± 0.13	0.55 ± 0.035	0.56 ± 0.027	0.98 ± 0.06
$MgCl_2$						
5 min	446 ± 30	7.31 ± 0.11	1.57 ± 0.22	0.48 ± 0.075	-	1.93[b] ± 0.12
30 min	460 ± 42	7.27 ± 0.10	1.82 ± 0.14	0.47 ± 0.074	-	2.59[b] ± 0.04
45 min	479 ± 39	7.25 ± 0.02	2.10 ± 0.39	0.57 ± 0.10	-	-
90 min	521 ± 38	7.13 ± 0.08	1.78 ± 0.15	0.46 ± 0.09	2.17[b] ± 0.058	2.99[b] ± 0.18

Values are means ± SEM; [a]Total plasma Mg. [b]Significantly different from control ($p<0.01$). N = 12.

gradient coils. After obtaining control ^{31}P-NMR spectra (prior to cocaine administration), each animal was removed from the NMR probe, a femoral vein was cannulated, and either $MgCl_2$ was administered (10 µmoles/min) at a constant infusion rate followed 45 min later by i.p. injection of cocaine HCl (5, followed by 30 mg/kg 2 hr. later) or a comparable volume of 0.9% NaCl. Each cocaine-or saline-injected animal was then returned to the NMR probe and repeat ^{31}P-NMR spectra were obtained at various intervals of time (*e.g.*, 3-120 min, or up until death had occurred). Control, naive animals were administered the cocaine HCl in the absence of Mg^{2+} infusion. Animals were autopsied upon death or sacrificed to determine whether subarachnoid or intracerebral bleeding had occurred. All animals identified as having hemorrhagic stroke exhibited, upon autopsy, 1-3 ml of blood in the subarachnoid space and/or brain.

The chemical shift difference between the α- and β-phosphoryl group resonances of ATP ($\delta_{\alpha\beta}$), along with a knowledge of the apparent K_d of MgATP (50 µmol/1 at pH 7.2, 37°C) under intracellular ionic conditions, was used to determine the concentration of $[Mg^{2+}]_i$ (4,10):

$$\theta = \frac{\delta^{cell}_{\alpha\beta} - \delta^{MgATP}_{\alpha\beta}}{\delta^{ATP}_{\alpha\beta} - \delta^{MgATP}_{\alpha\beta}}$$

$$[Mg^{2+}]_i = K_d^{MgATP}((1/\theta) - 1)$$

The K_d^{MgATP} was corrected for varying pH as needed. $\delta_{\alpha\beta}^{MgATP}$ = 1340 Hz and $\delta_{\alpha\beta}^{ATP}$ = 1748 Hz were utilized for calculations.

Intracellular pH (pH_i) was measured from the ^{31}P-NMR spectra by use of the following equation (4):

$$pH_i = 6.73 + \log(\delta_{obs} - 2.90\ V_p)/(5.70\ V_p - \delta_{obs})$$

where V_p is the ^{31}P Larmor frequency in MHz and δ_{obs} is the chemical shift difference between the Pi and P-creatine (PCr) resonances in Hz.

The [PCr]/[ATP] and [Pi]/[ATP] concentration ratios were calculated from the ratios of integrated areas and corrected for partial saturation of resonance intensities (4). Ion selective electrodes (ISEs) were utilized to measure plasma ionized Mg^{2+}, H^+, K^+ Na^+, and Ca^{2+} (11). Total plasma Mg was measured with a Kodak Ektachem DT60 Analyzer (11). Where appropriate, mean values ± S.E. were calculated and compared using paired or unpaired Student's t-test,; and ANOVA for multiple comparisons. Chi-square tests (and regression analyses) were also used. A P-value less than 0.05 was considered significant.

The animal experiments were conducted in accord with the highest standards of human aminal care and that the appropriate approval of the experiments has been obtained from the university committee dealing with this issue.

4. RESULTS AND DISCUSSION

Continuous intravenous infusion of 10 µmol/min of $MgCl_2$, in normal control rats, lowered mean arterial blood pressure (5-25 mmHg), but failed to the alter brain $[Mg^{2+}]$, pH , [PCr], [PCr]/[ATP] or $[P_i]$ up to 2 hr (Table 1). The regimen of $MgCl_2$ increased the plasma ionized Mg^{2+} 300% and total plasma Mg threefold over normal; plasma Ca^{2+}, Na^+, H^+ and K^+ were not altered, even after 2 hr of continuous Mg^{2+} infusion.

Table 2: Effects of cocaine in the presence and absence of magnesium infusion on brain $[Mg^{2+}]_i$, pH, and intracellular phosphometabolites

Group time-min	$[Mg^{2+}]_i$ µM	pH_i	[PCr]/[ATP]	$[P_i]$/[ATP]
Controls	466±35	7.24±0.03	1.89±0.14	0.61±0.06
$MgCl_2$ + Cocaine				
3-5 min	366±29	7.24±0.04	2.28±0.19	0.60±0.06
15 min	401±33	7.29 ±0.08	2.04±0.17	0.59±0.05
120 min	463±45	7.27±0.12	2.41±0.25	0.73±0.08
Cocaine Alone				
3-5 min	428±28	7.20±0.08	1.85±0.30	0.94±0.12[b]
15 min	292±37[b]	6.73-7.02[b]	1.48±0.19[b]	2.48±3.38[b]

Values are means ± S.E.M. [b]Significantly different from controls and $MgCl_2$ + Cocaine ($p < 0.05$). N = 8-16 each. Only N = 3 measurements of pHi and [Pi]/[ATP], after cocaine alone (at 15 min when most animals died), were made since the resonances for [Pi] were not well defined; hence the range of values for N = 3.

40% (8 out of 20) of animals died in the absence of Mg^{2+} infusion following cocaine administration. However, only 13% (4 out of 30, $p<0.05$) died with Mg^{2+} infusion, suggesting a better than 3-fold protection of Mg^{2+}. All animals which died, upon autopsy, exhibited intracranial and/or intracerebral bleeding (1-3 ml). Table 2 demonstrates that in protected animals neither $[Mg^{2+}]$, pH_i, [PCr]/[ATP], nor $[P_i]$/[ATP] fall when toxic and lethal doses of cocaine are administered 45 min after constant infusion of 10 µmol/min $MgCl_2$. However, animals that receive similar toxic doses of cocaine, in the absence of Mg^{2+} infusion, demonstrated initially a fall in the brain $[Mg^{2+}]_i$, followed by progressive falls in pH_i and [PCr]/[ATP] and an increase in $[P_i]$/[ATP] (Table 2).

Low basal brain $[Mg^{2+}]_i$ (275 ± 24 vs. 466 ± 35 µM) and low basal brain [PCr] (3.36 ± 0.35 vs. 4.26 ± 0.25 mM) were found to result in a three-fold increased incidence of stroke ($p<0.01$). A positive correlation ($r = 0.31$, $p<0.03$) between brain $[Mg^{2+}]_i$ and [PCr]/[ATP] was found. In view of such new data, it is possible that brain $[Mg^{2+}]_i$ and [PCr] may be useful as important predictors of susceptibility to hemorrhagic strokes.

These findings point to a vasospastic response in cerebral microvessels in response to cocaine, leading to vascular occlusion and intracerebral, as well as subarachnoid, bleeding set into motion by loss of cerebral vascular smooth muscle and neuronal $[Mg^{2+}]_i$. The associated loss of [PCr] and rise in [Pi] and $[H^+]_i$, indicating severe ischemia, would be consistent with this hypothesis. Mg^{2+} therapy prevents these events from taking place. It is known that Mg^{2+} normally either gates or has an action on Ca^{2+} entry and intracellular release of Ca^{2+} (12-14). Thus, depletion of $[Mg^{2+}]_i$ by cocaine would allow entry and intracellular release of Ca^{2+} causing contraction. Recently, we have shown that treatment of cerebral vascular smooth muscle cells with cocaine HCl (10^{-9} to 10^{-5} M) induces concentration-dependent rapid rises in free cytosolic Ca^{2+} (8). Loss of $[Mg^{2+}]_i$ appears to precede the rapid rise in $[Ca^{2+}]_i$ (7,8).

The fact that significant levels of [PCr] remain in the stroked animals, associated with subarachnoid bleeds, immediately following death, suggests that cocaine exerts differential effects and actions on various regions of the brain.

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