Biochimica et Biophysica Acta, 1111 (1992) 271–274

BBAMEM 70716

Rapid Report

Cocaine induces intracellular free Mg deficits, ischemia and stroke as observed by in-vivo ^{31}P-NMR of the brain

Burton M. Altura [a] and Raj K. Gupta [b]

[a] *Department of Physiology, State University of New York, Health Science Center at Brooklyn, Brooklyn, NY (USA)* and [b] *Department of Physiology and Biophysics, Albert Einstein College of Medicine, Bronx, NY (USA)*

(Received 9 September 1992)

Key words: Cocaine; NMR, ^{31}P-; Magnesium ion; Stroke; Ischemia; (Rat brain)

^{31}P-NMR spectroscopic studies were performed in vivo on brains of rats administered cocaine. Cocaine · HCl (1–5 mg/kg) administered systemically to lightly anesthetized rats resulted in significant and progressive deficits in whole brain intracellular free Mg ($[Mg^{2+}]_i$). Intracellular pH (pH_i) also fell in a progressive manner but only after a significant fall in brain $[Mg^{2+}]_i$ was noted. Both $[Mg^{2+}]_i$ and pH_i returned to normal in most rats. Brains of rats that exhibited stroke-like events, however, demonstrated continued intracellular acidosis associated with progressive loss of phosphocreatine and elevation of P_i up until death. These observations are consistent with the tenet that injection of cocaine can result in severe cerebral vasospasm, ischemia and rupture of cerebral blood vessels as a consequence of depletion of brain $[Mg^{2+}]_i$.

Despite its reputation as a 'fun' drug that can be used recreationally, use of cocaine is associated with an ever-growing number of aneurysmal subarachnoid hemorrhages, intracerebral hemorrhages, brain edema and occlusion-type strokes in human subjects [1–6]. Onset of symptoms can occur within minutes to hours after ingestion of cocaine. Recently, it has been stated that cocaine can result in serious central nervous system damage and major alterations in fetal brain structure, with subsequent neurologic, cognitive, and behavioral dysfunctions [7–11].

In most epidemiologic stroke studies, researchers have stressed rightly the strong correlation between hypertension and predisposition to stroke. However, this linkage in cocaine-induced strokes is not readily apparent [1–6]. While it is certain that cocaine facilitates strokes in the brain, and that it is a risk for stroke in individuals who have cerebrovascular disease [5,6], it is by no means certain how these strokes are brought about.

Experimentally, using direct in vivo microcirculatory studies on the rat brain, we have shown previously that cocaine can induce concentration-dependent contractions of cerebral arterioles and venules in situ [12,13]. This work has recently been confirmed in piglets and fetal lambs [14,15]. Magnesium ions (Mg^{2+}), but no other pharmacologic antagonist tested successfully prevent, and rapidly reverse, these cocaine-induced contractions of cerebral microvessels as well as prevent and attenuate the rupture of cerebral microvessels in vivo [13].

Recent, preliminary in situ observations, performed in our laboratory, on the rat brain indicate that perfusion of the cortical microcirculation with cerebral spinal fluid containing reduced extracellular Mg^{2+} ($[Mg^{2+}]_o$) can result in rapid and progressive spasms of arterioles and venules followed by rupture of venules and capillaries, leading to focal hemorrhages and brain edema [16]. These results are, thus, very similar to what we have observed with administration of cocaine in normal, Mg^{2+}-sufficient brain preparations. We, therefore, hypothesized that cocaine-induced reduction in cerebral vascular muscle and neuronal intracellular free Mg^{2+} ($[Mg^{2+}]_i$) might set into motion the cocaine-associated cerebral vasospasm resulting in hypoxia, ischemia and stroke.

Male Wistar rats, weighing 135–180 g, were anesthetized lightly with pentobarbital sodium (Nembutal, 3 mg/100 g, i.m.). After induction of anesthesia, each rat was placed in a General Electric Omega 400 WB spectrometer with a 9.4 T vertical bore magnet utilizing double-tuned ^{31}P/^{1}H RF coils [17]. Body temperature was maintained by keeping the magnet at 30–32°C and by placing a blanket around each animal. The animal was carefully accommodated in the NMR probe (with

Correspondence to: B.M. Altura, Box 31, SUNY-Health Science Center at Brooklyn, 450 Clarkson Avenue, Brooklyn, NY 11203, USA.

head pointing down) so that all of the brain was contained within the RF coil. In order to make certain the brain was positioned properly, we also obtained proton images using S50 gradient coils. After obtaining control ^{31}P-NMR spectra (prior to cocaine administration), each animal was removed from the NMR probe and carefully injected i.p. with doses of cocaine · HCl (100% purity, N.I.D.A., 1–5 mg/kg, dissolved in normal physiologic saline) shown previously to result in vasospasm and occassional rupture of cerebral, cortical micovessels in rat brain [12,13]. Each cocaine-injected animal was then returned to the NMR probe and repeat ^{31}P-NMR spectra were obtained at various intervals of time (e.g., 4–70 min, or up until death had occurred).

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/l at pH 7.2, 37°C) under intracellular ionic conditions, was used to determine the concentration of $[Mg^{2+}]_i$ [18–20]:

$$\phi = \frac{\delta_{\alpha\beta}^{cell} - \delta_{\alpha\beta}^{MgATP}}{\delta_{\alpha\beta}^{ATP} - \delta_{\alpha\beta}^{MgATP}} \quad (1)$$

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

The K_d^{MgATP} was corrected for varying pH [17,18]. In a typical ^{31}P-NMR spectrum of in vivo brain, the signal to noise ratio was 100 and the widths of the ATP

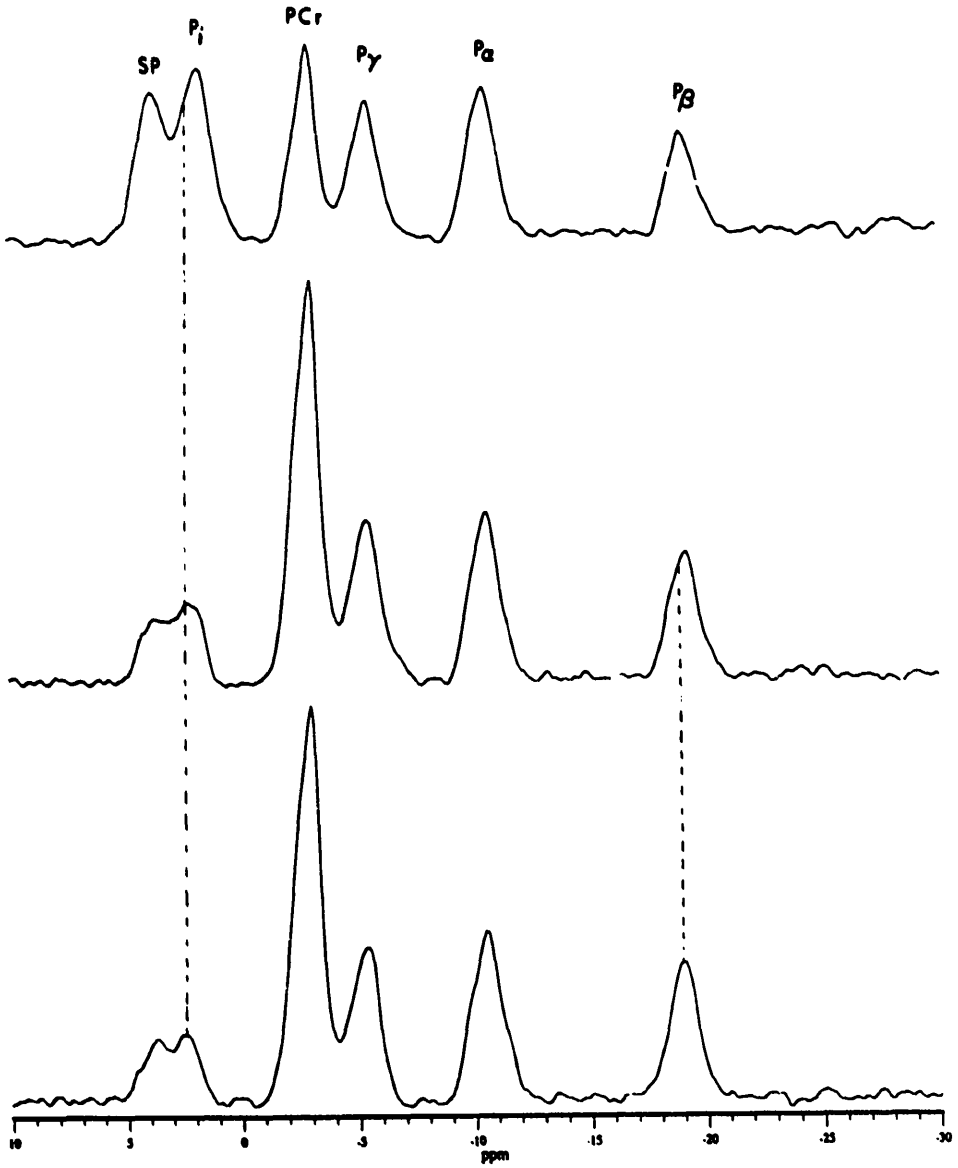

Fig. 1. ^{31}P-NMR spectra of rat brain showing cocaine-induced alterations in $[Mg^{2+}]_i$, pH_i, [PCr] and P_i. Spectra represent control animal (bottom tracing), 28 min after injecting cocaine·HCl, 5 mg/kg (middle tracing) and 50 min after injecting cocaine·HCl, 5 mg/kg (top tracing). ^{31}P resonances of phosphoryl groups of ATP (P_α, P_β, P_γ), phosphocreatine (PCr), inorganic phosphate (P_i) and sugar phosphates (SP) are labeled. The vertical lines show the positions of P_β and P_i resonances before cocaine admistration.

resonance lines were approx. 200 Hz. A Monte Carlo simulation was used to estimate the error of the NMR chemical shift measurement under these conditions and yields a standard deviation of 4 Hz.

Intracellular pH (pH_i) was measured from the ^{31}P-NMR spectra; the protonation state, hence chemical shift of the intracellular phosphate (pH_i) resonance, is pH-dependent and allows pH_i to be calculated by use of the following equation [19,21]:

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

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.

In some animals, the [PCr]/[ATP] and [Pi]/[ATP] concentration ratios were calculated from the ratio of integrated areas and corrected for partial saturation of resonance intensities. Where appropriate, mean values ± S.E. were calculated and compared using paired or unpaired Student's *t*-test. A *P*-value less than 0.05 was considered significant.

Of seven animals injected with 1–5 mg/kg of cocaine, six different brains consistently demonstrated significant reductions (35–78%, depending upon dose and animal) in measured $[Mg^{2+}]_i$; the peak drop in $[Mg^{2+}]_i$ (which was rat and time-dependent) averaging 420 μM or about a 57% loss in brain $[Mg^{2+}]_i$ (320 ± 22 μM in cocaine-treated vs. 747 ± 162 μM in controls, $P < 0.03$). In some animals, significant reductions in brain $[Mg^{2+}]_i$ were noted as little as 10 min after cocaine administration. A change in brain $[Mg^{2+}]_i$ always preceded significant alterations in pH_i, [PCr]/[ATP] and [Pi]/[ATP], irrespective of dose of cocaine · HCl. Animals that failed to demonstrate the latter bioenergetic alterations, exhibited restoration of brain $[Mg^{2+}]_i$ and pH_i within 50 min, probably a result of either cocaine metabolism or natural resistance.

Five of the seven cocaine injected brains demonstrated consistent drops in pH_i (7.12 ± 0.05 in cocaine-treated vs. 7.33 ± 0.06 in controls, $P < 0.03$), approx. 7–12 min after the initial $[Mg^{2+}]_i$ deficit was observed. Such observations would be consistent with the idea that cocaine-induced cerebral ischemia is preceded by deficits in brain $[Mg^{2+}]_i$.

Two of the seven cocaine-injected rats got into difficulty approx. 50–60 min after cocaine administration; one dying 10 min thereafter and the other remaining unconscious for almost 24 h. Such animals demonstrated stroke-like events upon removal of the calvarium; i.e., bleeding on the brain surface and often intracerebrally. Examination of the in vivo ^{31}P-NMR spectra of the stroked animals followed up until death (Figs. 1 and 2) revealed an interesting pattern over time, consisting of progressive loss in brain $[Mg^{2+}]_i$ (peaking at about 30 min) followed by progressive

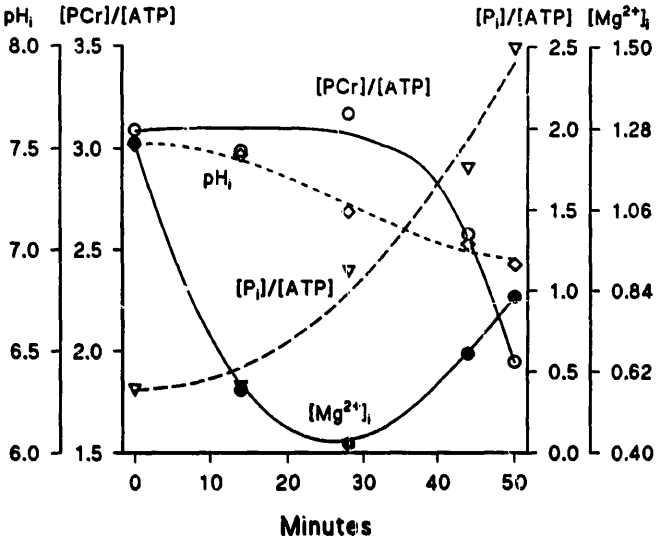

Fig. 2. Representative graphs showing time-dependent changes in $[Mg^{2+}]_i$ (●), pH_i (◇), [PCr]/[ATP] (○), and [Pi]/[ATP] (▽), in a rat brain, after injection of 5 mg/kg cocaine·HCl. $[Mg^{2+}]_i$ are expressed in units of mM.

reduction in pH_i indicative of intracellular acidosis due to ischemia. It is also apparent from these spectra that cocaine-induced brain ischemia, which lead to stroke-like events, causes progressive loss of PCr concomitant with elevation in [Pi].

These findings would be consistent with a vasospastic response in cerebral microvessels leading to vascular occlusion and/or intracerebral bleeding set into motion by a loss of cerebral vascular smooth muscle and neuronal $[Mg^{2+}]_i$. All contractile events in muscle tissue are mediated by an increase in the cytoplasmic level of free calcium ions (Ca^{2+}), and cocaine probably releases Ca^{2+} for contractility in cerebral vascular smooth muscle [22]. It is known that Mg^{2+} normally either gates or has an action on Ca^{2+} entry and intracellular release [23,24]. Thus, depletion of $[Mg^{2+}]_i$ by cocaine in cerebral vascular smooth muscle, and neuronal tissue, would allow uncontrolled entry and intracellular release of Ca^{2+} causing cerebral vasospasm. The progressive, irreversible rise in $[H^+]_i$ and [Pi] and the associated loss in PCr in stroked subjects would be consistent with this tenet. The apparent rebound of $[Mg^{2+}]_i$ in the stroked animals (Fig. 2), associated with elevation of the [Pi]/[ATP] ratio and concomitant reduction of the [PCr]/[ATP] ratio, is probably an effect of intracellular acidosis on $[Mg^{2+}]_i$. The fact that Mg^{2+} also stabilizes vascular endothelium [25], is an anticoagulant [25], and can prevent excessive neurotransmitter release as well as block the *N*-methyl-D-aspartate (NMDA) receptor [26] may bolster its natural anti-cocaine properties.

This work was supported in part by ADAMHA Research Grant AA-08674. We are grateful to Dr. Bella T. Altura for her helpful criticisms and comments.

References

1 Caplan, L.R., Hier, D.B. and Banks, G. (1982) Stroke 13, 869–872.
2 Mittleman, R.E. and Wetli, C.V. (1987) J. Forensic Sci. 32, 11–19.
3 Mangiardi, J.R., Daras, M., Geller, M.E., Weitzner, I. and Tuchman, A.J. (1988) Acta Neurol. Scand. 77, 177–180.
4 Klonoff, D.C., Andrews, B.T. and Obana, W.G. (1989) Arch. Neurol. 46, 987–993.
5 Levine, S.R., Brust, J.C.M., Futrell, N., Brass, L.M., Blacke, D., Fayad, P., Schultz, L.R., Millikan, C.H., Ho, K.-L. and Welch, K.M.A. (1991) Neurol. 41, 1173–1177.
6 Sloan, M.A., Kittner, S.J., Rigamonti, D. and Price, T.R. (1991) Neurology 41, 1358–1364.
7 Cohen, S. (1984) Psychol. Ann. 14, 747–749.
8 Dupont, T.L. (1985) J. Am. Med. Assn. 254, 2335–2337.
9 Lenman, L.B. (1987) Postgrad. Med. 81, 150–152.
10 Richardson, G.A. and Day, N.L. (1991) Neurotoxicol. Teratol. 13, 455–460.
11 Handler, A., Kistin, Davis F. and Ferre, C. (1991) Am. J. Epidemiol. 133, 818–825.
12 Altura, B.M., Altura, B.T. and Gebrewold, A. (1985) Fed. Proc. 44, 1637.
13 Huang, Q.-F., Gebrewold, A., Altura, B.T. and Altura, B.M. (1990) Neurosci. Lett. 109, 113–116.
14 Albuquerque, M.L. Kurth, C.D., Kim, S.J. and Wagerle (1991) Pediatr. Res. 29, 56A.
15 Covert, R.F., Schreiber, M.D., Torgerson, L.J. and Torgerson, R.W. (1992) Pediatr. Res. 31, 59A.
16 Altura, B.M., Gebrewold, A., Huang, Q.-F. and Altura, B.T. (1991) Clin. Res. 39, 394A.
17 Altura, B.M., Altura, B.T. and Gupta, R.K. (1992) Magnesium and Trace Elements 10, in press.
18 Gupta, R.K. and Moore, R.D. (1980) J. Biol. Chem. 255, 3987–3993.
19 Altura, B.M., Barbour, R.L., Reiner, S.D., Zhang, A., Cheng, T.P., Dowd, T.L., Gupta, R.K., Wu, F. and Altura, B.T. (1992) in Imaging in Alcohol Research, Monograph No. 21 (Zakhari, S. and Witt, E., eds.), pp. 235–272, USA Gov't Printing Office, NIAAA, Wash, DC.
20 Gupta, R.K., Gupta, P. and Moore, R.D. (1984) Annu. Rev. Biophys. Bioeng. 13, 221–246.
21 Jacobus, W.E., Pores, I.H., Lucas, S.K., Weisfeldt, M.L. and Flaherty (1982) J. Mol. Cell. Cardiol. 14 (Suppl. 3), 13–20.
22 He, G.-Q., Zhang, A., Altura, B.T. and Altura, B.M. (1992) FASEB J. 6, A986.
23 Turlapaty, P.D.M.V. and Altura, B.M. (1978) Eur. J. Pharmacol. 52, 421–423.
24 Altura, B.M., Altura, B.T., Carella, A., Gebrewold, A., Murakawa, T. and Nishio, A. (1987) Can. J. Physiol. Pharmacol. 65, 729–745.
25 Altura, B.M. (1988) Magnesium 7, 57–67.
26 Watkins, J.C., Krosgaard-Larsen, P. and Honoré, T. (1990) Trends Pharmacol. Sci. 11, 25–33.