Analysis of brain metabolism changes induced by acute potassium cyanide intoxication by ^{31}P NMR in vivo using chronically implanted surface coils

M. Decorps*+, J.F. Lebas*, J.L. Leviel*+, S. Confort†, C. Remy* and A.L. Benabid*

*Laboratoire de Médecine et Chirurgie Expérimentales et Comparées, UER de Médecine, Cermo, BP 53X, 38041 Grenoble Cédex, +Laboratoire de Résonance Magnétique, DRF, Centre d'Etudes Nucléaires, BP 85X, 38041 Grenoble Cédex, and †Laboratoire d'Electronique et Technologie de l'Informatique, MCTE, Centre d'Etudes Nucléaires, BP 85X, 38041 Grenoble Cédex, France

Received 19 January 1984

Chronic implantation of surface coils on the skull has been developed to record ^{31}P NMR spectra of the brain in unanesthetized rats. Intraperitoneal sublethal potassium cyanide doses induce strong and reversible changes in high-energy phosphate compounds in the brain, similar in part to those induced by ischemia. These effects are dose-dependent as far as phosphocreatine, inorganic orthophosphates and pH are concerned; ATP does not seem to be altered by KCN doses ranging from 3 to 5 mg/kg but starts decreasing at a dose of 6 mg/kg. The fraction of Mg^{2+} complexed ATP which could be estimated as about 90% was not affected by KCN intoxication. For high doses (6 mg/kg) a new peak, appearing on the upfield side of the inorganic phosphate peak, may correspond to an acidic compartment, the significance of which is discussed.

Surface coil *^{31}P NMR spectroscopy in vivo* *Potassium cyanide* *Rat brain* *Cytochrome oxidase*

1. INTRODUCTION

Nuclear magnetic resonance (NMR) studies have proven their ability to track metabolic pathways through biochemical analyses. The development of surface coils [1] significantly helped application of these methods to in vivo studies of metabolic phenomena [2–8]. ^{31}P NMR spectroscopy is specifically able to probe high-energy phosphate compounds. Chronically implanted surface coils ensure a stable geometry of the radiofrequency coil with respect to the observed brain, reproducible throughout subsequent experiments, and provide a way to hold the animal in a fixed position during the recording session without need of anaesthesia. We here used ^{31}P NMR spectroscopy with chronic surface coils to follow the time course of intracellular metabolic changes induced by specific inhibition of the respiratory chain by KCN, which blocks electron transport from cytochrome aa_3 to oxygen. Studies of the effects of KCN other than those performed on cellular or subcellular preparations in vitro [9–13] are few. Follow-up studies of the spontaneous reversal of the metabolic effects of KCN-induced cytochrome aa_3 blockade can only be achieved by non-invasive methods such as NMR spectroscopy.

2. MATERIALS AND METHODS

2.1. *Animals and surface coils*

Adult Sprague-Dawley rats (250–490 g) were anaesthetized with intraperitoneal thiopental (50 mg/kg) and placed on a KOPF stereotaxic frame and the surface coil was chronically implanted (fig.1). The animals were allowed to recover from surgery for several days. The position of the surface coil with respect to the brain was

Published by Elsevier Science Publishers B.V
00145793/84/$3 00

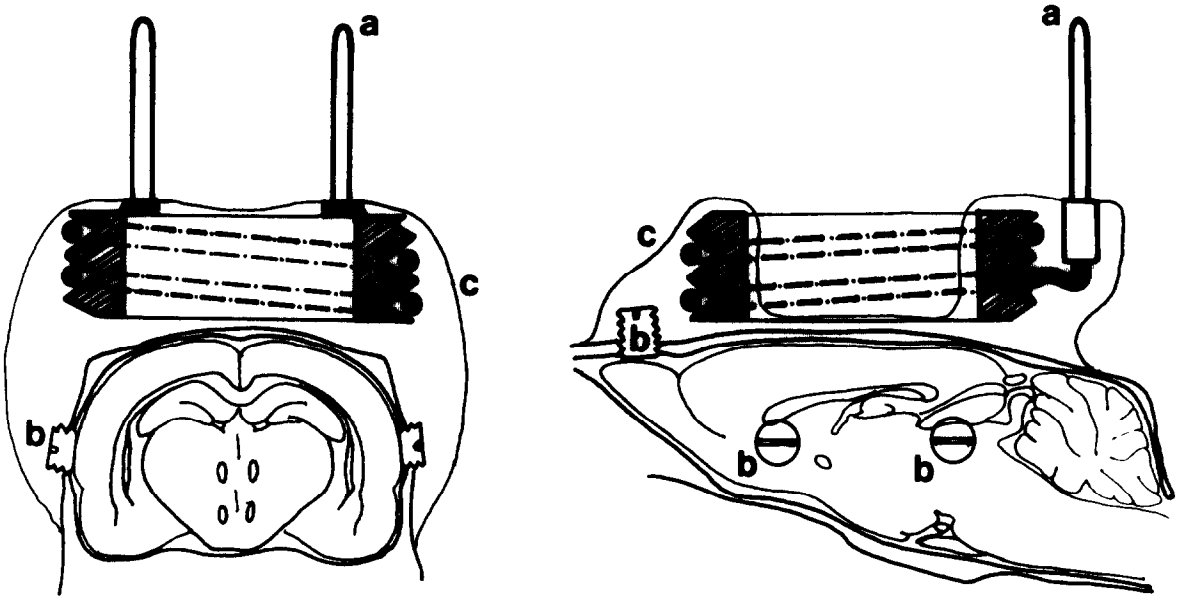

Fig.1. Chronically implanted surface coil. The surface coil is made of two turns of silver-coated copper wire in the helicoidal groove of a 20-mm diameter Teflon former (shaded area). The wire is soldered to two pins (a) perpendicular to the planes of the coil. Under general anesthesia, on a stereotaxic frame, the skull was exposed, including parietal surfaces. Five Teflon screws (b) were inserted into burr holes 3 mm in diameter, two in each parietal surface and the fifth on the midline over the olfactory lobes. The surface coil was positioned horizontally over the skull, its center above the middle point between lambda and bregma, the first turn of the wire being 1 mm from the skull. Dental cement (c) (methyl methacrylate) embeds both the screws and the coil. The skin is sutured around but not over the coil, to minimize blood contribution from extracerebral components.

kept perfectly unchanged throughout repeated experiments for periods of about 1 month. At the time of the experiment, the rats were briefly anaesthetized with diethyl ether and placed in a 70-mm diameter home-built NMR probe fitting the wide bore of a 4.7 T Oxford Instruments superconducting magnet. The male pins of the surface coil, perpendicular to the surface coil and skull planes, were plugged into the corresponding female connectors of the NMR probe. The axis of the surface coil was therefore kept perpendicular to the main axis of the supermagnet. A 19 G Teflon catheter was inserted into the peritoneal cavity, secured to the skin by a suture, and connected to a polyethylene tube, mounted on a syringe filled with a freshly prepared KCN solution (3, 4, 5 or 6 mg/ml in isotonic saline). Then the animal, when enclosed in the probe, was allowed to wake up.

2.2. *NMR spectra*

The probe, containing the animal, was inserted into the vertical wide-bore superconducting magnet of a Bruker CXP 200 spectrometer. Field homogeneity was shimmed on the proton NMR signal at 200 MHz. ^{31}P spectra were recorded at 80.98 MHz, with a sweep width of $\pm$ 4000 Hz. The pulse width of the radiofrequency pulses was 40 μs (180° at the center of the coil) [14]. Pulse delay was 1 s. Interpretable spectra were obtained within 150 s (150 scans), making it possible to follow the dynamics of metabolic changes with good temporal resolution. However, before KCN injection, 150-scan spectra were stored every 150 s over 30 min, to obtain by summation a high signal-to-noise ratio reference spectrum, and to assess statistical fluctuation of peak intensities. After intraperitoneal KCN injection, spectra were stored every 150 s over 90 min and difference spectra between them and the reference were displayed. The broad line ($\simeq$2400 Hz) due principally to the hydroxyapatite bound phosphorus atoms in the skull, but also attributed for a smaller part to the phospholipids in the brain [15], is removed by a

convolution difference technique using 400 Hz and 40 Hz line broadenings. Further increase in resolution is obtained if necessary by using a Gaussian multiplication. Chemical shifts are measured relative to phosphocreatine (PCr) taken as zero. Intracellular pH was calculated from the relationship

$$pH = 6.66 + \log\left(\frac{\delta P_i - 3.079}{5.57 - \delta P_i}\right)$$

(adapted from [6]) where δP_i is the chemical shift of the inorganic phosphate (P_i) peak. The binding of Mg^2 to ATP was calculated using the equation in [16], with adaptation of the constants to the rat body temperature (37°C). We assumed that such a quantitative model derived from Ehrlich ascites tumor cell suspensions may be legitimately used for calculations in the in vivo rat brain. The difference curves presented in this study are drawn from the values of the intensity of each peak, above the zero level of the spectrum, as given by the spectrometer software.

Because of the deconvolution procedures and of the overlapping of some peaks, we measure only semi-quantitative variations of the peak amplitudes, which is however sufficient for our purpose. The loss of accuracy so far introduced in our in vivo NMR measurements is compensated, from the biological point of view, by the opportunity afforded of following the evolution of a metabolic phenomenon, in an animal which serves as its own control.

3. RESULTS AND DISCUSSION

3.1. *Assignment of resonances of ^{31}P spectrum in normal rat brain*

The normal ^{31}P spectrum of unanaesthetized rat brain is shown in fig.2. Because of the broad lines of the in vivo spectrum, resonances from com-

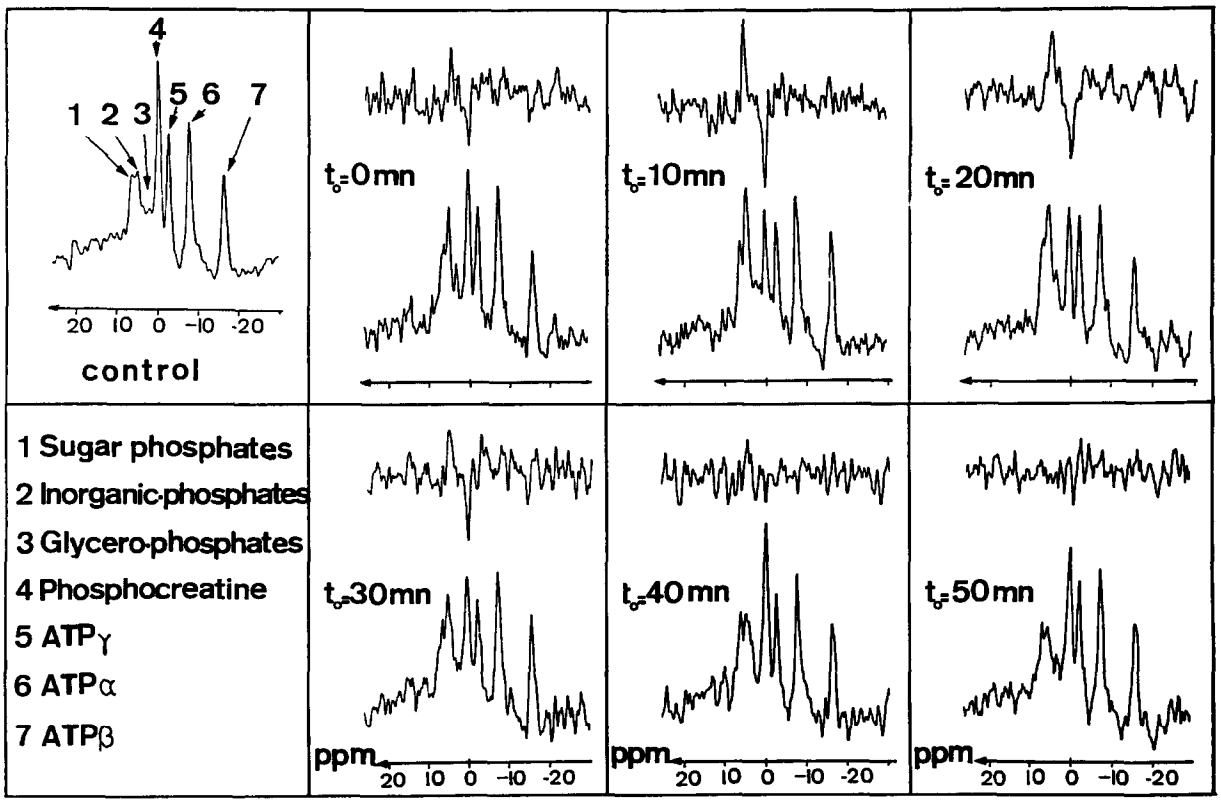

Fig.2. ^{31}P spectra of rat brain during 5 mg/kg KCN experiment. After a control spectrum (900 scans), injection was made at $t = 0$, and spectra were recorded for about 100 min. (a) Control spectrum (150 scans), (b) difference spectrum with control spectrum. Peak assignment: 1, sugar phosphate (SP); 2, P_i; 3, glycerophosphate (GP); 4, phosphocreatine (PCr); 5, γ-ATP; 6, α-ATP; 7, β-ATP. Each spectrum was accumulated during the time interval from t_o to $t_o + 2.5$ min.

pounds of comparable chemical shifts are indistinguishable from each other and participate in the same peak. Major ^{31}P resonances of energy metabolism cellular compounds in brain have been identified from tissue extracts [2,11] and whole brain [1,2,8]. The difference in the chemical shifts of the β-ATP resonance (2.12 ppm) between our observed in vivo value (-16.24 ± 0.10 ppm) and the value obtained from perchloric acid extracts of guinea pig brain (-18.33 ± 0.005 ppm) is due to almost total binding of ATP in vivo by Mg^{2+} [17–19]. The proportion of uncomplexed ATP was found to be 11% ± SD 3.4%. These values are in accordance with those in [17] and they may be compared to those calculated from perchloric acid extract [11], where 88% of ATP is not complexed.

3.2. *Metabolic changes induced by intraperitoneal injection of KCN*

The dose-effect relationship was investigated with 3 different doses (3, 4, 5 mg/kg) in 3 groups of 3 animals and a 6 mg/kg dose in one animal. In all cases the observed changes in NMR spectra were of the same type: the higher the dose, the longer the intensity and duration of the effects. Qualitative aspects and time courses of typical changes following intraperitoneal KCN administration are shown in fig.2,3. Within the first 150 s after injection, PCr starts decreasing drastically while P_i increases in similar proportions, as shown by difference spectra. In the meantime, tissue pH decreases to about 6.7 for a 6 mg/kg dose of KCN. Sugar phosphate and glycerophosphate peaks exhibit no significant changes at any KCN dose, while ATP, which decreases only for the 6 mg/kg dose, is not altered up to a dose of 5 mg/kg, which is known to produce 75% inhibition of cytochrome oxidase activity within 2 min [13]. The parallel experiments with freely moving rats injected with similar KCN doses show a good correspondence between the metabolic changes as shown by ^{31}P spectroscopy and behavioral alterations. The decrease in ATP after a 6 mg/kg dose (fig.3) is about 30–40% of its control value. For lower doses (3–5 mg/kg), the lack of variation in ATP and ADP contrasts with the changes observed during brain ischemia. While in myocardial ischemia PCr initially decreases alone, and ATP and ADP are altered only later when PCr has almost disappeared, it has been

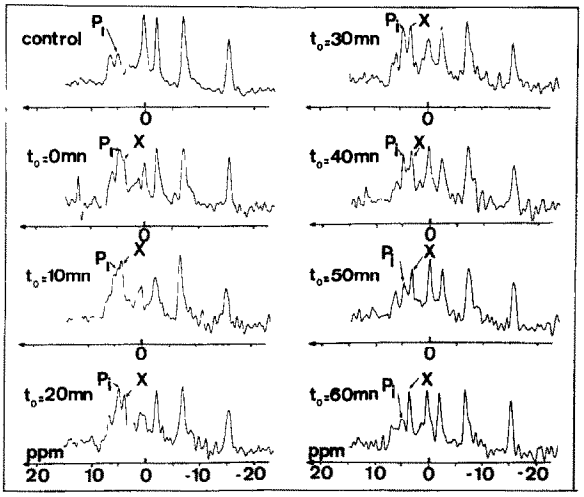

Fig.3. ^{31}P spectra of rat brain during a 6 mg/kg KCN experiment. Appearance of the peak X. The resolution of these spectra was enhanced by Gaussian multiplication. Each spectrum was accumulated during the time interval from t_o to t_o + 10 min.

reported [8,20] that this dissociation does not occur during brain ischemia, where PCr and ATP decrease together. This difference could be explained by the lower creatine kinase activity in brain ($200\ \mu mol \cdot g^{-1} \cdot min^{-1}$) than in heart ($600\ \mu mol \cdot g^{-1} \cdot min^{-1}$) or by compartmentalization of ATP [8]. Similar effects were also observed during bicuculline-induced seizures [21]: PCr was decreased by about 50%, glycogen, glucose and glucose 6-phosphate decreased by more than 50%, while lactate increased about 6-fold, but ATP, ADP and AMP levels were not changed. This lack of variation in ATP has been recently reported following hypoxia and bicuculline-induced seizures [6]. The dose-dependent effect on ATP changes observed here suggests that preservation of ATP levels at low-dose (3–5 mg/kg) KCN intoxications could be due either to a decrease of the backward flux of creatine kinase activity or to an insufficient impairment of cellular respiration by KCN, as compared to that induced by severe brain ischemia. This second hypothesis is in agreement with reported data on neonate rat brain ischemia where PCr and ATP fall coordinately at 37°C while ATP is practically constant and PCr decreases when ischemia is produced after reduction of brain temperature to 20°C which reduces brain metabolism [22]. If it turns out to be true,

this could provide a convenient means for prognostic evaluation in case of human brain ischemia.

After a 6 mg/kg KCN injection, the P_i peak increases and shifts upfield as shown in fig.3 and a satellite peak (X) appears on the upfield side of the P_i peak. The intensity and chemical shift of this X peak increase as a function of time. After 30 min, the X peak is higher than the P_i peak and its shift remains constant at about 3.3 ppm from the PCr reference. Peak X could be interpreted as a P_i peak corresponding to a tissue pH of 5.65, from an extracellular compartment of lower pH than the intracellular one, or could be due to a subset of cells undergoing a lethal process as may be suggested by the death of the animal a few hours after the end of the experiment. This 'dead cells hypothesis' would also account for the loss of ATP which is observed together with the appearance of peak X. Another interpretation would attribute this peak to glycerophosphates, ranging between 3 and 4 ppm from the PCr reference [11]. Whatever its origin, peak X seems to be related to the severity of tissue damage, and could therefore provide an index for prognostic evaluation. Additional experiments are required to confirm these observations and to test these hypotheses.

We have shown that ^{31}P NMR spectroscopy of the brain in vivo provides a convenient tool for studying changes in concentration of phosphorylated metabolites following a decrease in the rate of oxidative phosphorylation due to an inhibition of cytochrome oxidase by cyanide. This tool may be even more useful for toxicology, given that the determination of brain rather than liver cytochrome *c* oxidase activity is significant for the evaluation of antidotal therapy against cyanide intoxication [13].

ACKNOWLEDGEMENTS

We wish to acknowledge P.V. Vignais for helpful discussion, E. Geissler for correcting the English text, A. Abbadie and C. Arnaud for typing the manuscript, and A. Rousseau, M.F. Foray, E. Corral and G. Larmurier for excellent technical assistance. This work was in part supported by grants from the French Ministry of Industry and Research (82-M-1342), and from the Scientific Councils of the Medical School and of Grenoble I University.

REFERENCES

[1] Ackerman, J.J.H., Grove, T.H., Wong, G.G., Gadian, D.G. and Radda, G.K. (1980) Nature 283, 167–170.
[2] Chance, B., Nakase, Y., Bond, M., Leigh, J.S. and McDonald, G. (1978) Proc. Natl. Acad. Sci. USA 75, 4925–4929.
[3] Evanochko, W.T., Ng, T.C., Lilly, M.B., Lawson, A.J., Corbett, T.H., Durant, J.R. and Glickson, J.D. (1983) Proc. Natl. Acad. Sci. USA 80, 334–338.
[4] Grove, T.H., Ackerman, J.J.H., Radda, G.K. and Bore, P.J. (1980) Proc. Natl. Acad. Sci. USA 77, 299–302.
[5] Ng, T.C., Evanochko, W.T., Hiramoto, R.N., Ghanta, V.K., Lilly, M.B., Lawson, A.J., Corbett, T.H., Durant, J.R. and Glickson, J.D. (1982) J. Magn. Resonance 49, 271–286.
[6] Prichard, J.W., Alger, J.R., Behar, K.L., Petroff, O.A.C. and Shulman, R.G. (1983) Proc. Natl. Acad. Sci. USA 80, 2748–2751.
[7] Stevens, A.N., Iles, R.A., Morris, P.G. and Griffiths, J.R. (1982) FEBS Lett. 150, 489–493.
[8] Shoubridge, E.A., Briggs, R.W. and Radda, G.K. (1982) FEBS Lett. 140, 288–292.
[9] Albaum, H.G., Tepperman, J. and Bodansky, O. (1946) J. Biol. Chem. 164, 45–51.
[10] Schubert, J. and Brill, W.A. (1968) J. Pharmacol. Exp. Ther. 162, 352–359.
[11] Glonek, T., Kopp, S.J., Kot, E., Pettegrew, J.W., Harrison, W.H. and Cohen, M.M. (1982) J. Neurochem. 39, 1210–1219.
[12] Isom, G.E. and Way, J.L. (1976) Biochem. Pharmacol. 25, 605–608.
[13] Isom, G.E., Burrows, G.E. and Way, I.L. (1982) Toxicol. Appl. Pharm. 65, 250–256.
[14] Chaillout, J.J., Confort, S., Decorps, M. and Laval, M. (1983) 2nd Meet. Magn. Resonance Soc., San Francisco, Aug.16–19, book of abstracts, p 83.
[15] Cox, D.W.G., Morris, P.G., Feeney, J. and Bachelard, H.S. (1983) Biochem. J. 212, 365–370.
[16] Gupta, R.J. and Yushok, W.D. (1980) Proc. Natl. Acad. Sci. USA 77, 2487–2491.
[17] Gadian, D.G., Radda, G.K., Richards, R.E. and Seeley, P.J. (1979) in: Biological Applications of Magnetic Resonance (Shulman, R.G. ed) pp.463–535, Academic Press, New York.
[18] Cohn, M. and Hughes, T.R. (1962) J. Biol. Chem. 237, 176–181.

[19] Gupta, R.J. and Moore, R.D (1980) J. Biol. Chem. 255, 3987–3993.
[20] Thulborn, K.R., Du Boulay, G.H., Duchen, L.W. and Radda, G. (1982) J. Cereb. Blood Flow Metab. 2, 299–306.
[21] Harik, S.I., Busto, R. and Martinez, E. (1982) J. Neurosci. 2, 409–414.
[22] Norwood, W.I., Norwood, C.R., Ingwall, J.S., Castaneda, A.R. and Fossel, E.T (1979) J. Thorac. Cardio-vasc. Surg. 78, 823–830.