64. Acute Effects of Carbon Monoxide on Cerebral pH and Energy Metabolism Studied by Non-Invasive Phosphorus-31 NMR

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Introduction. Carbon monoxide (CO), a major atmospheric pollutants, is a leading cause of poisoning or intoxication, and there are many studies on its effects on humans.^{1)–3)} The extent of damage induced by carbon monoxide depends on the blood carboxyhemoglobin (COHb) level and the duration of exposure. The increase in the COHb level leads to lowered oxygen delivery to the tissues. The brain and heart muscles are very sensitive to this hypoxic condition, and are easily damaged. Metabolic acidosis is also significant manifestation in CO poisoning. A biochemical study revealed that the intracellular pH of rat brain dropped to 6.46 by inhalation of CO.⁴⁾ However, the time course of change in intracellular pH in brain during CO inhalation or exposure is still unknown, because the intracellular pH is difficult to measure non-invasively, especially in brain, with conventional biochemical techniques.

Phosphorus nuclear magnetic resonance spectroscopy is a non-invasive method for determining intracellular changes in high energy phosphates,⁵⁾ and it also gives us information about the change in pH.^{6)–8)}

We investigated the change in intracellular pH of rat brain during CO inhalation, as well as changes in high energy phosphates, using *in vivo* ³¹P NMR spectroscopy.

Materials and methods. Male Sprague-Dawley rats, each weighing about 170 g (169±3 g), were anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and intubated endotracheally. They were mechanically ventilated with a Ugo-Basile type 7025 ventilator after administration of pancuronium bromide (0.5 mg/kg, i.m.). To control the respiration of rat placed in the super conducting magnet, unusually long air tubes (1.6 m) were used. In the preliminary tests, animals were kept in the neighborhood of normoxic (PaO₂ 101.5±2.9 mmHg, n=4) and normocarbic (PaCO₂ 40.3±1.9 mmHg, n=4) condition while ventilated with a tidal volume of 2 ml, a minute volume of 200 ml, using 1.6 m long tubes.

NMR measurements were made with a JNM GSX-270 spectrometer system (JEOL, Japan). The surface coil (11 mm in diameter, five-turn) for NMR detection (Fig. 1(A)) was placed against the scalp of the animal (Fig. 1(B)). The spectra were obtained at an operating frequency of 109.14 MHz for phosphorus. Radiofrequency pulses of 20 μsec duration were delivered every 1.5 sec for NMR acquisition. It took 5 min to accumulate 200 scans for each measurement. Following collection of control data, inhalation of 5000 ppm CO (in air) was started. During the 40 min of inhalation, spectra were obtained every 10 min. Data were accumulated during the last 5 min of each 10 min. After the CO

Fig. 1. A: The surface coil for NMR detection (five-turn, 11 mm in diameter) used in this study. B: Experimental animal fixed on the probe. The animal's head is placed right above the surface coil.

Table I. Changes in Pi/PCr and intracellular pH of rat brain during and after CO (5000 ppm) inhalation (n=7)

Time (min)*	Inhaled gas	Pi/PCr	Intracellular pH
0	Air	0.56 ± 0.05	7.22 ± 0.16
10	5000 ppm CO in air	0.69 ± 0.10	7.19 ± 0.02
20		0.91 ± 0.19	6.93 ± 0.16
30		1.20 ± 0.30	6.59 ± 0.08
40		1.13 ± 0.28	6.61 ± 0.23
50	Air	0.63 ± 0.09	6.69 ± 0.19
60		0.59 ± 0.04	6.91 ± 0.27
70		0.61 ± 0.08	7.13 ± 0.13

Values are means $\pm$ S.D. * Time at the moment that the data accumulation was finished. Time 0 means the point when control data accumulation ended.

inhalation, the data for recovery period were also obtained in the same way for 30 min.

The intracellular pH (pH_i) was determined from the chemical shift of inorganic phosphate peak relative to phosphocreatine peak according to the following equation.⁹⁾ $\text{pH}_i = 6.77 - \log_{10} [2.51 / (\delta - 3.22) - 1.0]$, where δ is the chemical shift of inorganic phosphate (ppm). The ratio of signal intensities of inorganic phosphate and phosphocreatine (Pi/PCr) was also obtained for each spectrum.

Results and discussion. Seven of all ten animals tested survived throughout the experiment. One died in the course of CO inhalation, the other two after inhalation. Fig. 2(A–H) shows a typical sequential change in the ^{31}P *in vivo* NMR spectra of brain obtained non-invasively from one of the surviving animal at each stage of the experiment. Fig. 2(I) shows a typical spectrum from dead animal. Changes in Pi/PCr ratio and intracellular pH of brain for seven survivors during and after inhalation of CO are shown in Table I. CO inhalation gradually increased the Pi/PCr, and the ratio was almost normalized by ventilation with air after CO loading (Fig. 2(A–H), Table I). The intracellular pH

Fig. 2. The sequential change in ^{31}P *in vivo* NMR spectra of brain obtained from one animal during and after CO inhalation is shown in A-H (A, control; B, 10 min inhalation; C, 20 min inhalation; D, 30 min inhalation; E, 40 min inhalation; F, 10 min after inhalation; G, 20 min after inhalation; H, 30 min after inhalation). The spectrum shown in I is from another animal that died during CO inhalation. The assignment of peaks is: 1, ATP(β); 2, ATP(α)+ADP(α); 3, ATP(γ)+ADP(β); 4, phosphocreatine; 5, inorganic phosphate; 6, sugar phosphate.

significantly dropped from 7.22 ± 0.16 (control) to 6.59 ± 0.08 (30 min CO inhalation), and returned to 7.13 ± 0.13 (30 min post-inhalation). The pH value 7.22 obtained in our control is in agreement with that reported by Litt *et al.*⁸⁾ Interestingly, cerebral pH declined to 6.59 by CO inhalation, but it recovered to 7.13 within 30 min. This rapid recovery may be related to the lack of accumulation of CO in the tissues as Sokal *et al.*¹⁰⁾ reported. Although accurate evaluation of the cerebral pH in dead animals is difficult because of the absence of the phosphocreatine peak in the NMR spectrum, the intracellular pH of three animals that died during or after the CO inhalation in this study seems to be below 6.5, and this value is compatible with the result of a biochemical study with DMO (5,5-dimethyl-2,4-oxazolidinedione) method reported by Gonmori.⁴⁾

Delivoria-Papadopoulos *et al.*¹¹⁾ reported changes in cerebral energy metabolism during CO inhalation. However, we are the first to report the sequential change in intracellular pH of brain during and after CO inhalation.

Further studies are needed to determine the relationship between the change in intracellular pH of brain and blood COHb levels.

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