Effect of Cerebral Ischemia and Hypercapnia on Cerebral pH Studied with ^{31}P -NMR and Electrical Activity in Rat Brain

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Abstract The activity of electroencephalogram (EEG) and cortical somatosensory evoked potential (SEP) was suppressed during cerebral ischemia in rats subjected to the 4-vessel occlusion. Considerable variations were demonstrated in the decrease of phosphocreatine and ATP concentration during ischemia among the rats measured with ^{31}P -NMR, accompanied with cerebral acidification. Hypercapnia, induced in the rats studied by the inhalation of a gas mixture of 30–40% CO_2 , suppressed the activity of EEG and cortical SEP. The cerebral acidification observed during the ischemia was more severe than that under the hypercapnia, implying that cerebral acidification is one of the possible causes for the decrease in the electrical activity of the brain during ischemia.

Key words: cerebral pH, cerebral ischemia, hypercapnia, evoked potential, ^{31}P -NMR.

Cerebral ischemia is well known to depress the activity of the brain as demonstrated by electroencephalogram (EEG). NARUSE *et al.* (1984) demonstrated clearly that cerebral ischemia reduced ATP and phosphocreatine concentration accompanied with cerebral acidification during ischemia in rats using ^{31}P -NMR. In this study, we examined the effect of ischemia on the activity of cortex and brainstem, monitoring the somatosensory evoked potentials (SEP) and auditory brainstem responses (ABR) together with energy metabolism in rats with ^{31}P -

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NMR. Furthermore, to evaluate the cause of the EEG suppression during the ischemia, the effect of cerebral acidification on the activity of the cortex and the brainstem was examined under hypercapnia in rats with ^{31}P -NMR.

Preliminary results have been reported elsewhere (TAKANASHI *et al.*, 1987).

MATERIALS AND METHODS

Preparation for cerebral ischemia and hypercapnia. Wistar rats weighing 200–300 g were anesthetized with intraperitoneal injections of 50% urethane (1 mg/g body weight). After a midline skin incision on the rat heads, the skin and bilateral temporal and occipital muscles were carefully removed in order to eliminate the contribution of these muscles to ^{31}P -NMR spectra and to insert the electrodes extradurally for recording EEG and evoked potentials, as described below. The body temperature of the rats was maintained at around 37°C using a warming blanket.

In the ischemia model, the bilateral vertebral arteries were electrically cauterized at the level of the first cervical vertebra, and the common carotid arteries were bilaterally ligated by the use of surgical silk thread for 40 min (4-vessel occlusion: PULSINELLI and BRIERLEY, 1979) and then released to observe recovery from the cerebral ischemia. Due to difficulties encountered in measuring complete cauterization of the vertebral arteries and suppression of the blood flow, rats in which the EEG became flat within 1 min after the onset of ischemia and which stayed flat at the end of the 4-vessel occlusion were used for the experiments. Rats which died during the ischemic period were excluded from the analysis.

In the hypercapnia experiments, gas mixtures of CO_2 and O_2 were supplied through a canula inserted into the trachea under artificial ventilation. Respiration rates were fixed at 60–70/min, and the ventilation volumes were adjusted so that the cerebral pH of the rats ventilated with room air remained within the values obtained on rats respiring spontaneously. The CO_2 content was increased step by step from 10 to 40% CO_2 by adjusting the flow rates under constant pressure. The blood pH and gases were analyzed (Radiometer ABL30, Denmark) on the blood obtained from femoral arteries of another 4 rats after 15-min inhalation of each gas mixture. In the preliminary experiments, the inhalation of pure oxygen gas somewhat influenced the EEG activity, but did not completely suppress the activity observed at 30–40% CO_2 (described later).

EEG and evoked potentials recordings. EEG and evoked potentials, such as cortical somatosensory evoked potential (SEP) and auditory brainstem response (ABR), were recorded from rats using biophysical amplifiers with a bandwidth of 3 kHz. The amplitudes in the components of the evoked potentials were calculated as the maximum deviations from the baselines.

A forepaw of each rat was stimulated with needle electrodes with square waves (0.1 ms duration, 2 mA current, 3 Hz) just sufficient to cause movement of the forepaw. The corresponding cortical SEP was recorded with a 100 μs sampling

interval from the screw electrode (diameter, 0.8 mm) placed on the skull overlying the sensorimotor cortex of the contralateral hemisphere to the stimulated forepaw. A needle electrode inserted in the nose was used as a reference. The 128 responses were averaged with a microcomputer (NEC PC-9801, Japan).

Acoustic stimuli, of click sounds with an alternating phase of 0.1 ms square pulses, were delivered at 16 Hz through a headphone placed near the ears with an intensity of 85 dB. The corresponding ABR was obtained with a 20 μ s sampling interval from the screw electrode placed at bregma, and the reference electrode was placed at lambda. The ABR samplings were averaged with 1,024 times accumulation.

³¹P-NMR measurements on rat brain. ³¹P-NMR studies were carried out with a SMR-200 NMR (JEOL, Japan) with Fourier transformations operating at 80.4 MHz utilizing a wide-bore, superconducting magnet. In each study, the rat head was placed on a small surface coil (diameter, 11 mm) centered 11 mm occipital to the bregma in the NMR probe. These precautions ensured that only the contents of the skull were observed. A probe constructed to restrain a rat was vertically placed in the superconducting magnet, and the homogeneity of the static magnetic field was optimized by observing the water proton signal. The spectra, measured with 14 μ s radiofrequency pulses repeated every 2.0 s, were accumulated 400 times for 14 min. Field lock and proton decoupling were not employed.

Cerebral pH was obtained from the difference (σ ppm) of the chemical shift between inorganic phosphate (P_i) and phosphocreatine (PCr), using the following equation (SEO *et al.*, 1983):

$$\text{pH} = 6.80 + \log(\sigma - 3.15)/(5.73 - \sigma).$$

The chemical shift was represented as 0 ppm at the PCr signal, with positive shifts downfield.

In the ischemia models, NMR spectra and electrical activity of EEG and evoked potentials were alternately measured on the same rats, and the relative changes in PCr and ATP concentration in the rat brains were measured from the resonance areas of the PCr and β -ATP signals, respectively, after the subtraction of the broad components. In the hypercapnia experiments, the relative changes in PCr and ATP were measured from the peak heights of those signals, which was possible because NMR conditions such as shimming and tuning were not changed during the experiments. The values were represented as mean $\pm$ S.D.

RESULTS

The effect of ischemia on phosphorus metabolites and cerebral pH

³¹P-NMR spectra from a rat brain obtained by a surface coil attached to the rat head are shown in Fig. 1A. Each spectrum was accumulated over a period of 14 min. The spectra showed a high resolution portion superimposing a broad component (linewidth, about 25 ppm). The high resolution portion showed the

Fig. 1. ^{31}P -NMR spectra of a rat brain showing severe cerebral ischemia produced by the 4-vessel occlusion (A) and the electroencephalogram (EEG) (B). The signals from sugar phosphates (sugar-P), inorganic phosphate (P_i), phosphocreatine (PCr), the γ , α , and β phosphate groups of ATP, and cerebral pH calculated from the chemical shift of P_i are indicated in the figure. The spectra were taken at the time indicated to the left of the spectra. During the 4-vessel occlusion the concentrations of PCr and ATP decreased markedly, accompanied by cerebral acidification.

ATP, phosphocreatine (PCr, 0 ppm), inorganic phosphate (P_i), and sugar phosphates (sugar-P) signals. The chemical shift of P_i was 5.08 ppm relative to PCr and the corresponding pH was 7.3 at the preischemic stage. The average cerebral pH value was 7.2 ± 0.1 , calculated from the chemical shift of P_i (4.99 ± 0.05 ppm, $n = 31$), on the control rats; rats with bilateral vertebral artery cauterization were included in these figures because there was no significant difference in the pH from the rats without the operation.

Blood flow suppression, not only from the carotid arteries but also from the vertebral arteries, was necessary to produce changes in the ^{31}P -NMR spectra during ischemia. As shown in Fig. 1, ischemia produced by the 4-vessel occlusion caused marked decrease in PCr and ATP with a corresponding increase in P_i . Cerebral pH

Fig. 2. ^{31}P -NMR spectra of a rat brain, showing mild ischemia produced by the 4-vessel occlusion (A), the EEG (B), the cortical somatosensory evoked potentials (SEP) (C), and the auditory brainstem responses (ABR) (D). The spectra and the electrical activities were taken sequentially at the times indicated to the left of the figure. During the 4-vessel occlusion, flat EEG activity was observed without any decrease in ATP. The SEP, composed of 4 cortical components (P_1 , N_1 , P_2 , N_2), disappeared during the ischemia and reappeared after recirculation through the carotid arteries. The later component (N_2) was delayed markedly in recovery and the amplitude increased 14 h later in the rat (the vertical scale in this case is reduced to half that of the others). The ABR components (P_1 , P_2 , P_3 , P_4), particularly the latter components (P_3 , P_4), were shown to be delayed in latency but the amplitudes did not decrease during the ischemia.

Table 1. Effects of ischemia on the relative concentrations of phosphorus metabolites, the cerebral pH, the relative amplitudes of the cortical somatosensory evoked potentials (SEP), and the auditory brainstem responses (ABR) in the rat brain.

Exp.No.	Cerebral			SEP		ABR	
	PCr	ATP	pH	P ₂	N ₂	P ₁	P ₄
1	0.2	0.5	7.3→6.5	0	0	1.1	1.2
2	0.9	1.1	7.3→6.8	0	0	2.0	1.6
3	0.7	0.8	7.2→6.7	0	0	2.0	1.9
4	0.7	0.9	7.2→6.6	0	0	1.4	1.5
5	0.5	0.8	7.2→6.7	0	0	0.8	1.2
Mean	0.6	0.8	7.2→6.7	0	0	1.5	1.5
S.D.	±0.3	±0.2	±0.1±0.1	±0	±0	±0.5	±0.3

The cerebral pH values presented are the preischemia values and the values they changed to, as obtained from the ³¹P-NMR spectra measured in the middle of the 40-min ischemia. The concentrations of phosphocreatine (PCr) and ATP and the amplitudes of SEP and ABR components are expressed as relative values of the preischemia values obtained from each rat.

decreased to 6.5. After the blood flow was allowed to recirculate through the bilateral carotid arteries, the PCr and ATP concentrations and the cerebral pH value recovered rapidly; however, flat EEG during ischemia did not recover even 1.5 h later (Fig. 1B). These results agree with those reported by NARUSE *et al.* (1984).

Figure 2 shows the effect of ischemia on another rat. A slight decrease in PCr and cerebral acidification was observed without the decrease in ATP. We could not rule out the possibility of incomplete suppression of blood flow through the vertebral arteries cauterized electrically or the blood vessel anastomosis. A flat EEG pattern was, however, observed in the rat during the ischemia.

The effects of ischemia on the 5 rats which showed flat EEGs during the ischemia are summarized in Table 1. Ischemia reduced the cerebral pH to 6.7 ± 0.1 from 7.2 ± 0.1 in the control group and the concentrations of PCr and ATP to 0.6 ± 0.3 and 0.8 ± 0.2 times of those of the control group, respectively, on average obtained from 5 rats. There were considerable variations in the decreases in the PCr and ATP values in the rats examined, as demonstrated in Fig. 1 (Exp. No. 1) and Fig. 2 (Exp. No. 2).

The effect of ischemia on electrical activity

To clarify the vulnerability of the cerebral cortex and brainstem, cortical SEP was examined together with ABR during and after ischemia, as shown in Fig. 2. The cortical components of SEP disappeared completely during the ischemia. The early component (N₁) measured 1 h 20 min after the start of recirculation, which

represents the response of the primary neurons in the somatosensory cortex (SHAW and CANT, 1981), reappeared with delayed latency; however, the later component (N_2), representing the polysynaptic components in the cortex, did not recover at this time. The suppression of EEG and cortical SEP indicated that cerebral cortex was quite sensitive to ischemia.

On the other hand, the ischemia caused only a slight delay in the latency of the early components (P_1 , P_2) of ABR representing the response of the acoustic nerve and a marked delay in that of the later components (P_3 , P_4) representing the response from the pons and/or the midbrain (HUANG, 1980). The amplitudes of these components were, in most cases, increased during the ischemia, as summarized in Table 1. These results indicated that the electrical activity of brainstem was not significantly suppressed by the ischemia in the 4-vessel occlusion model. These electrophysiological results are in agreement with the results reported by PULSINELLI and BRIERLEY (1970) stating that the blood flow to the brainstem was preserved probably via the anterior spinal artery in the 4-vessel occlusion models. Contradictorily, the delayed latency in ABR indicated that the 4-vessel occlusion did affect the brainstem.

The effect of hypercapnea on EEG and evoked potentials

Figure 3 shows the EEG activity, the cortical SEP, and the ABR of a rat under hypercapnia. The measurements were obtained after the inhalation of each gas mixture for at least 15 min under artificial ventilation, and CO_2 gas content was increased step by step from 10 to 40%. Cardiac arrhythmia appeared occasionally at 30–40% CO_2 .

The EEG activity began to decrease at 20% and disappeared at 30–40%, and the cortical SEP, especially the late components, began to decrease at 10% and disappeared at 40%. The ABR persisted with delayed latency of the components during the hypercapnia. Similar results were obtained with 3 other rats. Although mild hypercapnia (10% CO_2) has been reported to have complex effects on cortex, depression and excitation (KRNEVIĆ *et al.*, 1965), our results confirmed the suppression of cortical activity during severe hypercapnia (30–40% CO_2) and that the activity of the brainstem was more resistant to hypercapnia than was the cortical activity in the brain.

The effect of hypercapnea on cerebral pH and phosphorus metabolites of rat brain

The time course of the spectral changes during hypercapnia induced by the inhalation of CO_2 are shown in Fig. 4. Three spectra, measured sequentially at each gas mixture, revealed that the second and third spectra were nearly identical, indicating that a steady state was reached within the measurements of the first spectra, a period of 14 min. The third spectra were used for the analysis. The results obtained from 4 rats are summarized in Table 2, together with the blood gas analysis.

Hypercapnia induced by the inhalation of 10, 20, 30, and 40% CO_2 caused

Fig. 3. Effect of hypercapnia on EEG (A), the cortical somatosensory evoked potentials (SEP) (B), and the auditory brainstem responses (ABR) (C) on a rat brain. The electrical activities were measured at least 15 min after the inhalation of each gas mixture from air to 40% CO_2 under artificial ventilation. Hypercapnia induced by 30 and 40% CO_2 suppressed the EEG activity and cortical SEP.

Fig. 4. Effect of hypercapnia on the ^{31}P -NMR spectra of a rat brain. Hypercapnia induced by the inhalation of 10, 20, 30, and 40% CO_2 caused acidification of cerebral pH from 7.2, which was the control value with air, to 7.1, 7.0, 6.9, and 6.8, respectively, accompanied with a decrease in PCr and the corresponding increase in P_i without decrease in ATP.

cerebral acidification from 7.2, which was the control value with air, to 7.1, 7.0, 6.9, and 6.8, respectively, accompanied with a decrease in PCr and no significant change in ATP.

The relative changes in cerebral pH and phosphorus metabolites during the hypercapnia agreed qualitatively with those reported by SIESJÖ *et al.* (1972), although they reported slightly acidic values of cerebral pH calculated by the bicarbonate-carbonic acid method and from the creatine kinase equilibrium. LITT *et al.* (1985) also reported no significant decrease in ATP even at 70% CO_2 using ^{31}P -NMR on rat brains.

When cortical activity was suppressed at 40% CO_2 hypercapnia, the corresponding cerebral pH was 6.8, and was accompanied with no significant decrease in ATP. Therefore, the cerebral acidification below 6.8 observed during the ischemia would be the probable cause for the suppression of cortical activity during the ischemia.

Table 2. Effects of hypercapnia on the relative concentrations of phosphorus metabolites and cerebral pH in rat brain and on the arterial pH, P_{aCO_2} , and P_{aO_2} .

	Cerebral (n=4)			Arterial (n=4)		
	PCr	ATP	pH	pH	P_{aCO_2}	P_{aO_2}
Air	1.0	1.0	7.22 ± 0.04	7.40 ± 0.05	30 ± 5	100 ± 11
10%CO ₂	0.8 ± 0.1	0.9 ± 0.2	7.11 ± 0.05	7.07 ± 0.04	81 ± 13	346 ± 19
20%CO ₂	0.8 ± 0.1	1.0 ± 0.2	6.97 ± 0.03	6.90 ± 0.03	114 ± 17	345 ± 12
30%CO ₂	0.7 ± 0.1	0.9 ± 0.2	6.93 ± 0.04	6.80 ± 0.03	169 ± 20	297 ± 54
40%CO ₂	0.6 ± 0.1	1.0 ± 0.1	6.83 ± 0.04	6.71 ± 0.02	215 ± 21	302 ± 50

The concentrations of phosphocreatine (PCr) and ATP are expressed as relative values of those obtained from each rat respiring air. The blood gases, P_{aCO_2} and P_{aO_2} (partial pressure of CO₂ and O₂ in mmHg), and pH were analyzed from another group of 4 rats. The values are mean $\pm$ S.D.

DISCUSSION

The electrical activity, as shown by EEG and cortical SEP, was suppressed during the ischemia. NARUSE *et al.* (1984) demonstrated clearly that the suppression of EEG activity during the ischemia was due to the reduction of ATP in rat brain, coinciding with the report stating that the decrease in ATP was the cause of the rapid failure of synaptic transmission in brain slice (YAMAMOTO and KUROKAWA, 1970). NARUSE *et al.* (1984) also reported that all of the rats studied showed a marked reduction of ATP during ischemia. In our study, there were, however, considerable variations in the decrease in PCr and ATP among the rats examined (Table 1). The possible explanation for the discrepancy was that the cauterization of vertebral arteries in this study might have been insufficient for complete suppression of the blood flow, as evaluated only from the flat EEG activity shown during the 4-vessel occlusion. The rats which died during the ischemia might have had severe ischemic patterns as shown in Fig. 1, and the constraint of the rat placed vertically in the superconducting magnet as used in this study might have caused death during the ischemia.

During the ischemia one rat showed no decrease in ATP and only a slight decrease in PCr accompanied with cerebral acidification (Fig. 2). Several other studies have also reported depression of EEG activity during anoxia or hypoxia in the absence of a fall in ATP (SCHMAHL *et al.*, 1966; DUFFY *et al.*, 1972; HILBERMAN *et al.*, 1984). LESNICK *et al.* (1986) also reported that ATP remained unchanged and only PCr reduced at the time of failure of the cortical SEP on cats subjected to hypotension following bilateral carotid ligation. These findings lead to the suggestion that the decrease in ATP was not specifically responsible for the rapid attenuation of neuronal activity during ischemia.

One possible explanation for the apparent lack of relationship between the ATP level and the neuronal activity was the localized decrease in ATP near sites of high energy consumption, which would be undetected at macroscopic levels. LIPTON and WHITTINGHAM (1982) reported that using hippocampal slices exposed to an elevated potassium buffer, transmission was compromised prior to a decrease in ATP in the slices as a whole during hypoxia; however, ATP concentration in the molecular layer was lowered by the time inhibition of the synaptic transmission began, in which the synapses of the pathway occurred. Therefore, we could not rule out the possibility of undetected microscopic decreases in ATP during the ischemia in rat brain.

Metabolic changes were observed during the ischemia, a decrease in PCr and cerebral acidification, and these might be the cause of the electrophysiological deficiency. BESSMAN and CARPENTER (1985) proposed a PCr energy shuttle system, in which PCr plays a role not only as an energy reservoir to buffer changes in energy demand but also as an energy carrier to ensure an adequate energy supply under extreme conditions such as hypoxia and ischemia. Figure 2, however, suggests that the slight reduction in PCr (0.9 times that of the control group) would not be a possible cause for the complete suppression of cortical activity, because the slight reduction in PCr (0.8 times that of control) observed during hypercapnia (10–20% CO₂) did not accompany the complete suppression of cortical activity.

Although we could not completely rule out the possibility that energy failure was the cause of the suppression of the cortical activity because of the variations in the decreases of ATP and PCr, the possibility of cerebral acidification was also examined as a cause of the suppression. All of the rats showed cerebral acidification below 6.8 during the ischemia. The hypercapnia produced by 30 or 40% CO₂ inhalation suppressed the cortical activity and the cerebral pH was 6.9 and 6.8, respectively. The degree of the acidification (pH 6.8–6.5) observed during the ischemia was sufficiently lower. Therefore, cerebral acidification is a possible cause for the suppression of EEG activity and cortical SEP during cerebral ischemia. In fact, brain lactic acidosis has been reported to impair postischemic recovery of EEG and SEP (REHNCRONA *et al.*, 1981). LESNICK *et al.* (1986) also suggested that the elevated lactate in white matter blocked the conduction pathway and abolished the cortical SEP.

Although there is no direct evidence to support the explanation that acidosis is a cause of suppression of neuronal activity, several explanations are possible. The excess of protons produced by acidosis during ischemia and hypercapnia have to be removed from the intracellular compartment in order to maintain normal pH. One of the mechanisms responsible for regulating intracellular pH is the Na⁺/H⁺ exchange system driven by the sodium gradient. This exchange system has been found in brain synaptosomes (SAUVAIGO *et al.*, 1984). Activation of this system increases intracellular sodium, which might suppress the neuronal activities. As another explanation, the synthesis of acetylcholine in the brain has been reported to be impaired even without decreases in ATP during mild hypoxia

(GIBSON and BLASS, 1976), and ATP-supported uptake of acetylcholine by synaptic vesicles was found to be suppressed with pH levels remaining acidic (ANDERSON *et al.*, 1982). The decrease in pH has also been shown to inhibit depolarization-induced Ca^{2+} entry in synaptosomes (NACHSHEN and BLAUSTEIN, 1979). These findings suggest that cerebral acidosis causes transmission failure. However, these explanations remain to be elucidated in *in vivo* experiments. In conclusion, severe cerebral acidification would be one of the possible causes of the suppression of the electrical activity in cortex during ischemia.

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