

***In Vivo* ³¹P- Nuclear Magnetic Resonance Studies on Energy Metabolism of Ischemic Gerbil Brain: Comparison between Unilateral and Bilateral Common Carotid Artery Occlusion Models**

Masaru NAGASHIMA, Tatsuhiko YUASA and Tadashi MIYATAKE

Department of Neurology, Brain Research Institute, Niigata University, Niigata, 951 Japan

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Summary. The changes of cerebral energy state under bilateral and unilateral common carotid artery occlusion-reflow models in Mongolian gerbils were studied using *in vivo* ³¹P nuclear magnetic resonance spectrometry (³¹P-MRS).

All ten gerbils used in the bilateral common carotid artery occlusion-reflow model showed relatively uniform ischemic patterns, i.e., depletion of phosphocreatine (PCr) and adenosine triphosphate (ATP) and increase of inorganic phosphate (Pi) followed by relatively uniform recovery pattern of PCr, ATP and Pi.

Among 20 gerbils used in the unilateral common carotid artery occlusion-reflow model, 6 animals displayed the ischemic pattern but 14 animals showed no significant changes during ischemia. The ischemic pattern recovered more rapidly in the unilateral ischemic group than in the bilateral one.

Although the Mongolian gerbil has used customarily in the study of brain ischemia, the unilateral common carotid artery occlusion model showed significant inter-individual variation concerning the change of brain energy state during ischemia.

While the results of this study are essentially predictable from the prior works using classic biochemical methods, they are useful in the sense that we demonstrate the complete time course of changes in metabolites in the same animal.

INTRODUCTION

Recent advances in *in vivo* ³¹P nuclear magnetic resonance spectrometry (³¹P-MRS) bring us new considerations about patho-physiological aspects concerning alterations of high energy phosphate com-

pounds in the experimental cerebral ischemia^{3,7,8}. This method allows a monitoring of the cerebral energy state *in vivo*.

On the other hand, experimental models of cerebral ischemia using Mongolian gerbils have been widely taken because the ease with which cerebral infarction can be introduced.

This paper aimed to study the changes of cerebral energy state under cerebral infarction using *in vivo* ³¹P-MRS on the gerbil, and to demonstrate the usefulness of this new technique.

MATERIAL AND METHODS

In vivo ³¹P-NMR spectrometry: This was performed in a JEOL JNM GX270 spectrometer (bore diameter, 54 mm; magnetic field, 6.25 T; ³¹P resonant frequency, 109.25 MHz). The optimal measurement condition was investigated previously⁸. The magnetic field homogeneity was shimmed by the proton signal in the brain. The NMR signals were detected with the surface coil of a 4-turn copper wire coil of 12-mm in diameter. 13 μsec of 90 degree pulse width was chosen and the repetition time was set at 2 sec. Spectra were produced by fourier transformation of averaged free-induction decays. A spectrum was obtained with averaging 200 scans, required 400 sec. A capillary containing a small amount of hexamethyl phosphoramide (HMPA), with a chemical shift of about 28.5 ppm away from the position of the PCr, was placed on the opposite side of the objecting surface of the coil. It allowed us to calculate the changing values of

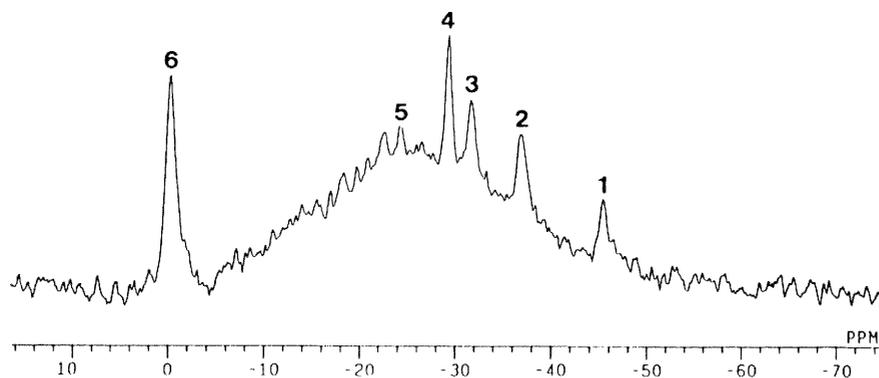


Fig. 1. ^{31}P -MRS from an intact gerbil brain. Peak assignments are as follows: 1, beta-ATP; 2, alpha-ATP; 3, gamma-ATP; 4, phosphocreatine (PCr); 5, inorganic phosphate; and 6, hexamethyl phosphoramide (external reference)

the phosphate compounds in the brain as a function of the height of each signal compared to that of HMPA⁸.

Experimental animals: Adult gerbils of either sex weighing from 60 to 100g were used. Anesthesia was induced with 3% halothane with oxygen following intraperitoneal administration of pentobarbital 35 mg/kg. After making a cervical midline incision, surgical threads were passed around either bilaterally or only on the right common carotid artery to enable subsequent transient occlusion. Immobilized animals were placed on a probe maintaining the head of the animal in a fixed upright position on the center of the surface coil, to detect the signals from the forebrain. In the case of the right common carotid artery occlusion model, the surface coil was placed on the right forebrain. After preocclusive spectra were observed, the common carotid artery was occluded from outside of the magnet by pulling the threads down using a sinker weighing 10g for each thread. After a 30 min occlusion period, the thread was removed and resumption of blood flow was ascertained by visual inspection. Spectra were obtained consecutively after occlusion and throughout the restoration period.

RESULTS

1. Normal ^{31}P -MRS: A typical ^{31}P -NMR spectrum of the forebrain of an anesthetized gerbil is shown in Fig. 1. The signals were assigned according to the chemical shift of each peak from previous reports^{3,7,8}. Peaks 1, 2, and 3 correspond to the signals from beta-,

alpha-, and gamma-phosphates of ATP respectively. Peak 4 is from PCr, Peak 5 is attributable to Pi, and peak 6 to external reference (HMPA). Chemical shifts are expressed in parts per million (ppm). The concentrations of each phosphorus compound were calculated from the value of the ratio of signal height of each phosphorus compound to that of HMPA. The large baseline hump derives from the phosphorus in the cranial bone¹.

2. Bilateral model (ten gerbils): Fig. 2 shows the changes of the ^{31}P -MRS from a gerbil brain the course of bilateral common carotid arteries occlusion-reflow model. The signal heights of both PCr and ATP had progressively decreased but the level of Pi had significantly increased during occlusion ("the ischemic pattern"). On the other hand, after resumption of arterial flows the pattern was reversed; increment of levels of PCr and ATP and decrement of levels of Pi ("the recovery pattern").

Figs. 3A, 3B, and 3C showed changes in the levels of PCr, ATP, and Pi respectively in 10 gerbils. After this we use the vertical scale for a hundred as a peak height of preocclusive state for each phosphorus compound in figures. Figs. 3A and 3B showed complete depletion of PCr and ATP and ATP respectively at the end of the occlusion period in all 10 animals. In contrast, Fig. 3C showed marked elevation of Pi during the occlusion period. Although the changes in the levels of PCr, ATP, and Pi were relatively uniform during occlusion, inter-individual differences in the changes of phosphorus compounds were larger after the reflow period than during occlusion. So these three figures, 3A-C, taken together showed that there was a uniform ischemic pattern during occlu-

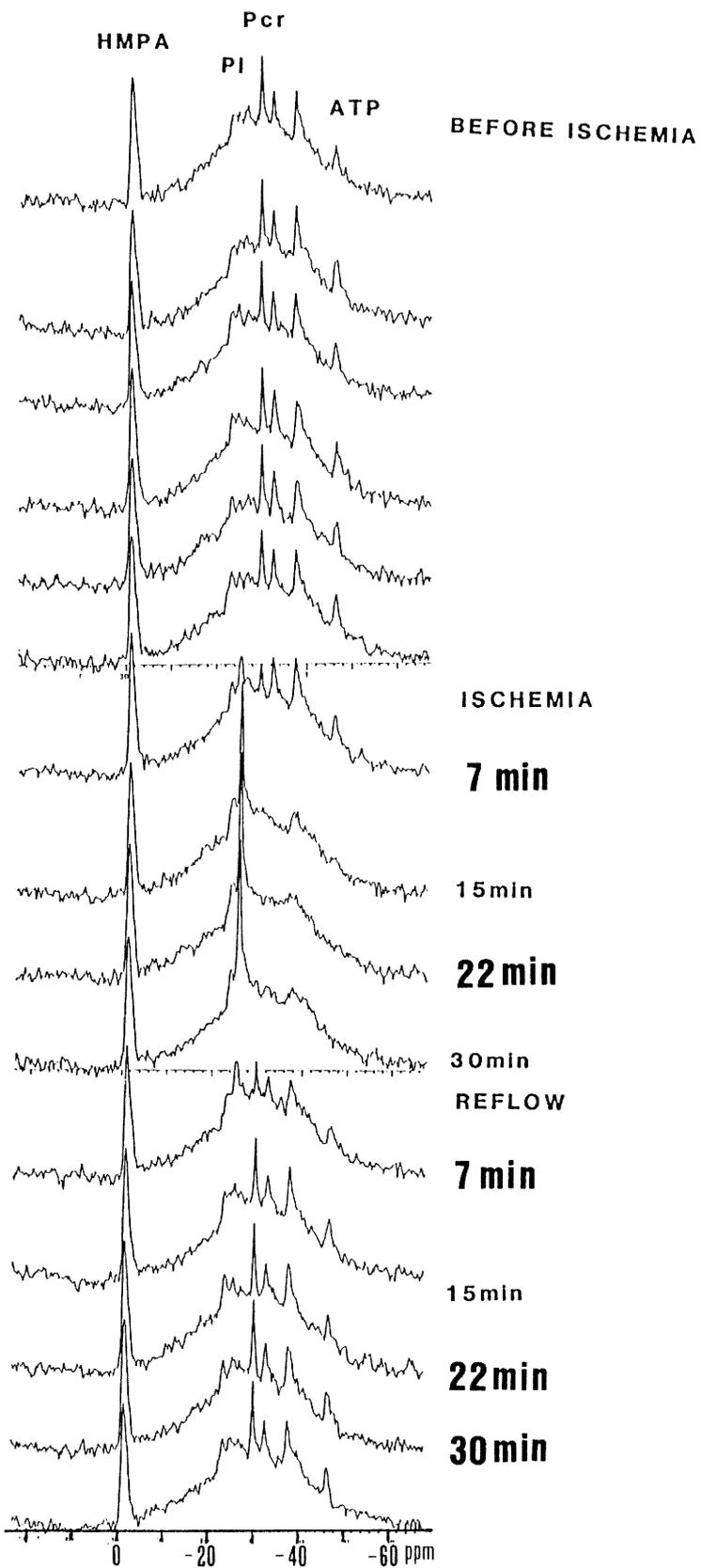
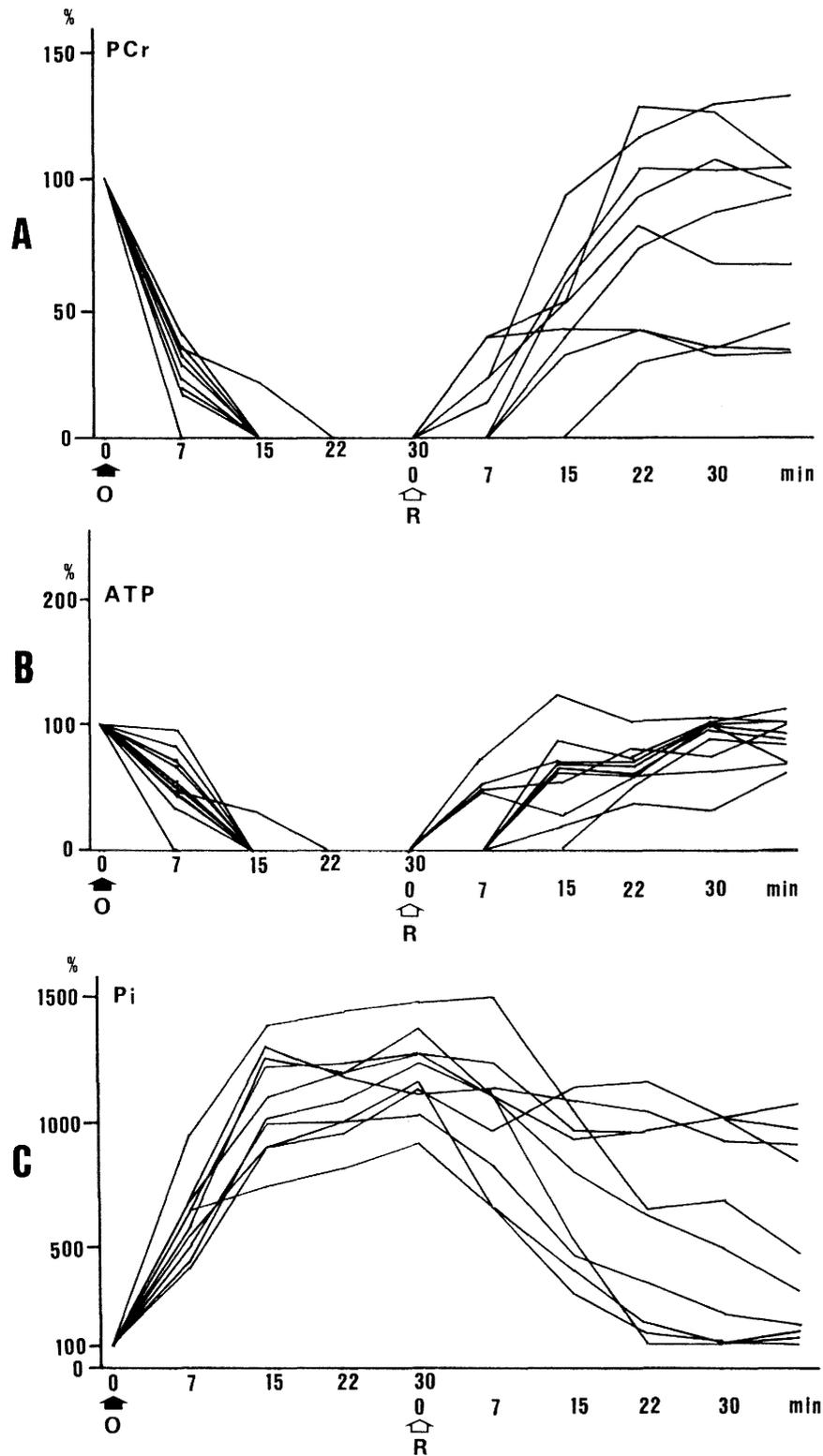
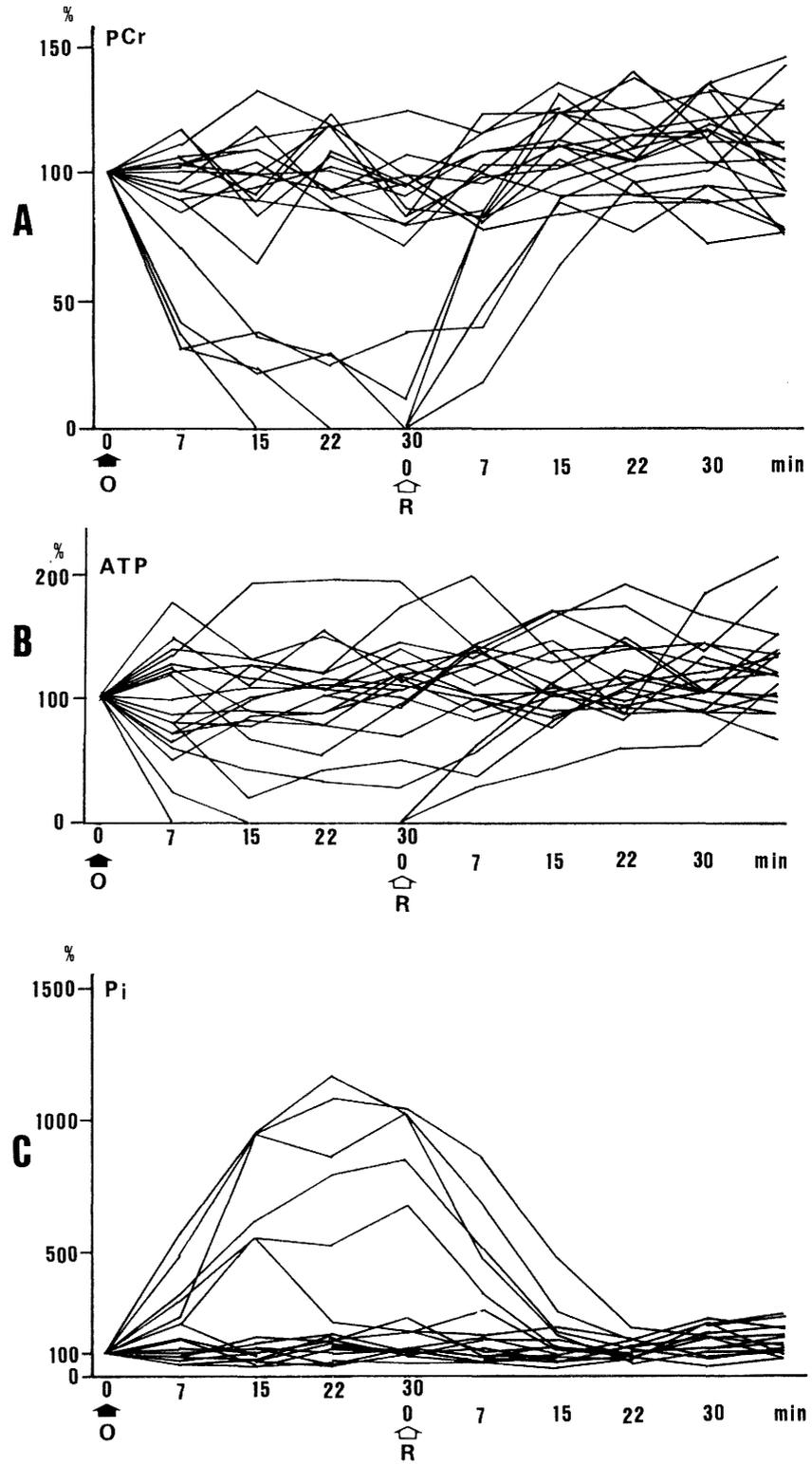


Fig. 2. The changes of the ³¹P-MRS in a gerbil brain during the course of bilateral common carotid arteries occlusion-reflow are shown.



Figs. 3A-C. Chronological changes of PCr(3A), ATP(3B), and Pi(3C) levels in the bilateral common carotid arteries occlusion-reflow model (10 gerbils). O: time of occlusion, R: time of reflow.



Figs. 4A-C. Chronological changes of PCr(4A), ATP(4B), and Pi(4C) levels in the unilateral common carotid artery occlusion-reflow model (20 gerbils). O: time of occlusion, R: time of reflow.

sion but a considerably variable recovery pattern after the reflow period among 10 gerbils in the bilateral common carotid arteries occlusion-reflow model.

3. Unilateral model (20 gerbils): ^{31}P -MRS changes in the right forebrain of the right common carotid artery occlusion-reflow models are shown in Figs. 4A, 4B, and 4C as Pi, PCr, and beta-ATP respectively. During occlusion, 14 animals showed no ischemic change and the other 6 showed various intermediate ischemic changes. These ischemic changes tended to recover more rapidly to the preocclusive state than that of the bilateral model. Among these three parameters, PCr, ATP and Pi, the levels of Pi showed the greatest change, whereas the levels of ATP showed the smallest one.

DISCUSSION AND CONCLUSION

The results of experiments presented here could be summarized as follows:

1. Bilateral common carotid arteries occlusion-reflow model animals showed a relatively uniform ischemic pattern (decrement in PCr and ATP, and increment in Pi) during the occlusion period in all cases. On the other hand, considerable inter-individual variations were noticed in the recovery modalities of cerebral energy levels during the reflow period.

2. Seventy-five percent of the unilateral occlusion-reflow model animals showed no ischemic change; 25% of them showed ischemic change and their recovery was more rapid than those of the bilateral model. These results described above had been predictable from previous works.

In 1966, Levine and Payan⁵ first introduced cerebral infarction of gerbils by bilateral and unilateral common carotid artery occlusion. Thereafter, investigations about gerbil's vascular anatomy revealed the absence of the posterior communicating artery^{2,6}. Many aspects of experimental cerebral ischemia were then investigated in this animal including energy states⁴. These classic biochemical analyses of energy metabolites such as ATP, PCr, and Pi sacrificed animals, and therefore information about chronological changes of these metabolites in the same animal had never previously been given. In addition, these classic biochemical methods may possibly under- or over-estimate the levels of metabolites during preparation of materials. Indeed, microwave radiation, one of the popular preparation methods used in classic biochemical analysis, was claimed to cause a decrease in the PCr/ATP ratio⁹.

Kobayashi et al. reported changes in gerbil brain energy metabolites under the bilateral carotid occlusion model using a classic biochemical method⁴. They showed almost complete depletion of ATP and PCr within 5 min, when our method demonstrated these compounds values required 15 min to reach their nadir. This difference could be explained by the loss of these compounds during preparation in the classic biochemical method.

In vivo ^{31}P -MRS may monitor the cerebral energy state chronologically on the same individual. Observations of *in vivo* ^{31}P -MRS on the experimental cerebral ischemia were recently reported by several authors. Thulborn KR et al.⁷ and Delpy DT et al.³ showed changes of ^{31}P MRS under cerebral ischemia. Although they showed a typical change of ^{31}P spectra after resumption in only one animal, the reproducibility and the inter-individual variations of the experiments were scarcely pointed out. We demonstrated here the reproducibility and inter-individual variations during and after transient cerebral ischemia in gerbils. These findings may presumably attributed to the gerbil's characteristic arterial anatomy.

Even though the results demonstrated in the present study essentially correspond to what is predictable from prior works using classic biochemical methods, they are valuable and useful in the sense that the changes in the metabolites concerned are recorded during the entire time course of the experiment using one and the same animal.

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