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High Energy Phosphate Metabolism in Experimental Permanent Focal Cerebral Ischemia: An In Vivo ^{31}P Magnetic Resonance Spectroscopy Study

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Summary: Relative levels of phosphate metabolites in the brain were examined in vivo by ^{31}P magnetic resonance spectroscopy (MRS) in 50 Sprague-Dawley rats before, during, and after induction of focal permanent cerebral ischemia. After acquisition of baseline spectra, rats were subjected to injury within the core of the MR spectrometer, and ^{31}P spectra were collected for 60 min after injury: in 7 rats, permanent, acute focal cerebral ischemia was induced (ischemia group); in 6 rats, mild hypoxia (FiO_2 14%) was induced at the time of the ischemic insult and was maintained for 20 min (ischemia-hypoxia group); in 6 rats, mild hypoxia (FiO_2 14%) only was induced for 20 min (hypoxia group). Control studies were performed in 25 rats. Cerebral intracellular pH, calculated from the chemical shift of inorganic phosphate (Pi), decreased immediately after injury in the ischemia and ischemia-hypoxia groups. The first ^{31}P spectrum obtained after injury was characterized by an increase in Pi and a decrease in phosphocreatine (PCr) in the ischemia and ischemia-hypoxia groups; these changes in spectra were significantly greater in the ischemia-hypoxia group. No significant changes in adenosine triphosphate (ATP) were found in either group. Within 60 min of occlusion, ^{31}P spectra returned toward baseline spectra in both ischemia-hypoxia and ischemia groups. No significant changes were seen in spectra of rats subjected to hypoxia alone. These results confirm that ^{31}P MRS is a sensitive measure of early changes of high energy metabolites in focal cerebral ischemia. The return of spectra toward baseline values within 1 h of injury despite the presence of permanent ischemic damage, however, suggests that caution should be used in attempts to interpret "recovery" of ^{31}P MRS. **Key Words:** Focal cerebral ischemia—Magnetic resonance spectroscopy—Phosphate metabolites—^{31}P.

Cerebral ischemic events frequently lead to hospitalization and may cause disabling morbidity and death. The National Center for Health Statistics (1986) estimated that the annual incidence of cerebral ischemia caused the loss of over 250,000 years of potential life in the United States in 1985. Moreover, the high incidence of cerebral ischemia in young people (Grindal et al., 1978) has led to research directed toward sensitive tests for early diagnosis that may allow more focused therapies (Spetzler et al., 1985; Batjer et al., 1986). The effects of cerebral infarction have been classified as transient ischemic attacks (TIAs), reversible ischemic neurologic deficits (RINDs), and complete stroke (Ogawa et al., 1985). This classification is retrospective, however, and in many instances, it is difficult to anticipate the clinical course of each patient in the period immediately after the onset of symptoms. The ability to detect possible changes in cerebral metabolism in the early phase of these pathologic entities might provide a better insight into eventual outcome.

High energy phosphate metabolism has been measured in vitro using biochemical techniques (Hossman et al., 1973; Ponten et al., 1973; Siesjo et al., 1973; Levy and Duffy, 1977; Carter et al., 1983). As these techniques require many steps and cannot be used clinically, methods to measure metabolic states noninvasively in vitro have been

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Abbreviations used: CCA, common carotid artery; MCA, middle cerebral artery; MRS, magnetic resonance spectroscopy; RIND, reversible ischemic neurologic deficits; PCr, phosphocreatine; Pi, inorganic phosphate; SP, sugar phosphate; TIA, transient ischemic attacks.

sought. Recent advances in magnetic resonance spectroscopy (MRS) allow a noninvasive study of brain metabolism in vivo (Chance et al., 1978, 1979, 1980; Roberts and Jardetzky, 1981; Dely et al., 1982; Gadian, 1982; Cady et al., 1983; Prichard et al., 1983; Gyulai et al., 1985). Experimentally, a temporary (15–60 min) ischemic-hypoxic insult to the brain is characterized by a depletion of high energy metabolites, with a subsequent "recovery" of ^{31}P MRS after the cessation of the noxious stimulus (Naruse et al., 1984*a;* Miyake, 1984; Horikawa et al., 1985; Litt et al., 1986). Before in vivo MRS can be used clinically, however, it is essential to define the importance and limitations of the spectra obtained. We have investigated the use of MRS to monitor high energy metabolism after permanent focal cerebral ischemia in rats and report our results here.

MATERIALS AND METHODS

Surgical procedure

The experimental procedure followed NIH guidelines and was approved by the UCSF Animal Experimentation Committee. Fifty adult male Sprague-Dawley rats, weighing 350–400 g, were anesthetized with an intraperitoneal injection of chloral hydrate (4% in saline; 35 mg/100 g body weight). Approximately 3 ml were used for induction, and 0.5 ml was given every 20 min after the beginning of the MRS experiments to maintain adequate anesthesia. Polyethylene catheters (PE-50) were introduced into the left femoral artery to monitor arterial blood pressure and gases and into the peritoneal cavity to administer chloral hydrate.

The left temporoparietal region was shaved and then prepared with betadine. A 2-cm curved vertical incision was made midway between the lateral margin of the left orbit and the external auditory canal. The temporalis muscle was elevated from the skull, and the inferotemporal fossa was exposed. Using an operating microscope and a saline-cooled dental drill, a 5-mm craniectomy was made, with care taken not to damage the zygomatic bone and the mandibular nerve. Rats then underwent a surgical procedure to allow occlusion of the middle cerebral artery (MCA) with the rat's head within the magnet. Ischemia was produced in two steps. First, the MCA was occluded by coagulation 5 mm distal to the point at which it is crossed by the inferior cerebral veins; this procedure alone does not cause cerebral infarction. Second, a snare was placed around the common carotid artery (CCA); pulling on the snare produces focal ischemia involving the distribution of the MCA. The procedure can be performed within the magnet. With permanent cartoid occlusion, this technique produces a 100% rate of infarction and an infarction size of 20 ± 3% (SD) of the coronal section for rats euthanized 24 h after the onset of ischemia (Germano et al., unpublished results). The rostral two-thirds of the crista frontalis was smoothed, and the temporoparietal bone was thinned over the left hemisphere with a dental drill, leaving a thin shell of bone over the dura where the MRS surface coil was to be positioned. Before spectra were obtained, rats were intubated and mechanically ventilated (FiO_2 30%; Harvard small animal ventilator). Mean arterial blood pressure (MABP) was continuously recorded. Arterial blood gases were monitored at each spectrum acquisition.

Study groups

Rats were divided into four groups. Focal cerebral ischemia was induced inside of the magnet in 7 rats (ischemia group). Mild hypoxia (FiO_2 14%; Ohio Oxygen Monitor 401, Airco Inc., WI, U.S.A.) was induced at the time of the ischemic insult and continued for 20 min in 12 rats (ischemia-hypoxia group). Mild hypoxia only (as above) was induced in 6 rats (hypoxia group). Twenty-five normal rats also were studied.

MRS technique

^{31}P spectra were obtained in a custom-designed 5.6 tesla cryogenic spectrometer with a bore diameter of 10.2 cm. Rats were mounted in a cradle that supported them in the lateral position. A 7 × 11 mm oval, two-turn, balance-matched surface coil tuned to the ^{31}P resonance frequency (95.808 MHz) was placed directly against the thinned skull over the left temporal fossa. A "saturated spectrum" was obtained in all rats before injury and was considered to be the baseline spectrum. Nine "saturated spectra" were acquired sequentially 3, 6, 9, 12, 15, 21, 30, 45, and 60 min after the injury. "Saturated spectra" were measured with 800-ms interpulse delay and 224 acquisitions; each spectrum was generated from about 3 min of data collection. A simple one-pulse excitation experiment was preceded by a low power presaturation pulse of 600 ms to minimize the broad component from bone phosphates and phospholipids (Gonzales-Mendez et al., 1984). Signal intensity of phosphocreatine (PCr), inorganic phosphate (Pi), sugar phosphate (SP), adenosine triphosphate (ATP), and total phosphate (P_{tot}) were determined by curve-fitting using the Nicolet program NTCCAP (Nicolet 118-data processor; Nicolet Instrument Corporation, Madison, WI, U.S.A.). Intracellular pH was calculated from the chemical shift (δ) of the Pi resonance peak relative to the PCr resonance peak with the following equation (James, 1984):

$$pH = 6.72 + \log (\delta - 3.27)/(5.69 - \delta)$$

Neuropathologic studies

After spectra were obtained, rats were given additional anesthesia (3 ml) and killed by perfusion of 10% buffered formalin. Brains were removed and fixed in formalin for 24 h. Two-millimeter–thick brain slices were embedded in paraffin, and 7-μm–thick coronal sections were stained with hematoxylin and eosin (H & E). Histopathologic evaluation was done by light microscopy.

Data analysis

Data are expressed as mean ± standard error (SE). Ratio of PCr/P_{tot}, Pi/P_{tot}, $\beta\text{-ATP}/P_{tot}$, and PCr/Pi were calculated to allow quantification of the status of high energy brain metabolites in tissue in each spectrum. As spectra were obtained under conditions that did not allow complete relaxation of the ^{31}P nuclei between radio frequency pulses that were necessary to maintain the efficiency of data collection, these ratios do not provide a measure of relative metabolite concentration as reported elsewhere (Ackerman et al., 1980), but do provide useful ratios for comparing spectra (see Discussion). The peak of β-ATP was chosen for the measurement of ATP concentration because peaks of α- and γ-ATP were contami-

nated with signals for adenosine diphosphate (ADP) and NADH, respectively (Prichard et al., 1983). Intragroup statistical analysis was performed by evaluating changes in a particular metabolite over time (null hypothesis: there is no difference between times) by a one-way analysis of variance (ANOVA) for repeated measures. Intergroup statistical analysis was performed by one-way ANOVA factorial (null hypothesis: there is no difference between groups at a given time). Bonferroini's correction was used for the post-hoc tests. The exact determination was done by the Fischer PLSD test and the Scheffe test for homogeneity of variance. A probability (p) of less than 0.05 was considered to be significant.

RESULTS

Physiologic values

Sequential changes in MABP and arterial blood gases are listed in Table 1. During mild hypoxia (FiO_2 14%), the lowest values in P_aO_2 were 48 ± 3 and 48 ± 5 in the ischemia-hypoxia and hypoxia groups, respectively. Intergroup analysis showed no difference in the increased P_aO_2 and MABP values between the hypoxia and ischemia-hypoxia groups. No significant changes in physiologic values were observed in the ischemia group.

Changes in MRS

Sequential changes in phosphate metabolites before and up to 60 min after injury in the control, hypoxia, ischemia, and ischemia-hypoxia groups are summarized in Table 1. Figure 1 shows a "saturated" ^{31}P spectrum (interpulse delay 800 ms) of a control rat.

Mean values for the baseline intracellular pH

TABLE 1. *Sequential changes in high energy phosphate metabolites measured by ^{31}P MRS and mean arterial blood gases and mean arterial blood pressure*

Group[a]	pH	P_{CO_2} (mm Hg)	P_{O_2} (mm Hg)	MABP (mm Hg)	HR	PCr	Pi[b]	ATP[b]	pH_i
Control rats	7.44 ± 0.06	31 ± 4	137 ± 5	102 ± 5	370 ± 35	17.3 ± 0.9	5.4 ± 0.3	12.1 ± 0.6	7.33 ± 0.07
Baseline									
H	7.43 ± 0.07	31 ± 2	138 ± 7	98 ± 2	370 ± 30	17.9 ± 0.9	5.1 ± 0.7	10.7 ± 0.9	7.18 ± 0.05
I	7.45 ± 0.07	30 ± 3	137 ± 9	103 ± 2	373 ± 13	18.4 ± 1.3	4.9 ± 0.7	12.0 ± 1.0	7.21 ± 0.07
I-H	7.46 ± 0.02	32 ± 3	139 ± 1	103 ± 4	393 ± 42	18.7 ± 0.9	5.1 ± 0.5	11.9 ± 0.9	7.19 ± 0.04
3 min after injury									
H	7.37 ± 0.04	35 ± 1	62 ± 9	78 ± 19	335 ± 15	17.6 ± 1.2	7.2 ± 1.1	10.4 ± 0.9	7.09 ± 0.13
I	7.41 ± 0.02	31 ± 2	130 ± 6	101 ± 2	360 ± 20	16.9 ± 1.5	6.4 ± 1.2	10.7 ± 0.5	6.95 ± 0.12
I-H	7.37 ± 0.03	37 ± 2	54 ± 8	73 ± 15	347 ± 27	15.0 ± 1.4	11.8 ± 2.1	10.8 ± 1.0	6.98 ± 0.08
6 min after injury									
H	7.39 ± 0.04	36 ± 1	58 ± 8	68 ± 13	333 ± 17	17.1 ± 1.2	5.4 ± 0.6	11.8 ± 1.2	7.19 ± 0.09
I	7.42 ± 0.06	31 ± 1	135 ± 6	93 ± 4	378 ± 19	15.6 ± 0.8	10.0 ± 2.3	10.2 ± 0.7	7.20 ± 0.07
I-H	7.34 ± 0.05	38 ± 2	53 ± 7	58 ± 15	325 ± 18	15.1 ± 1.2	9.8 ± 1.9	11.4 ± 0.9	7.11 ± 0.10
9 min after injury									
H	7.38 ± 0.09	36 ± 4	51 ± 5	70 ± 14	320 ± 16	17.1 ± 1.3	5.4 ± 1.6	11.9 ± 0.8	7.15 ± 0.10
I	7.47 ± 0.04	29 ± 2	132 ± 2	91 ± 6	370 ± 15	17.3 ± 1.3	8.2 ± 1.2	9.7 ± 0.5	7.24 ± 0.12
I-H	7.34 ± 0.01	37 ± 3	52 ± 5	60 ± 10	318 ± 21	15.7 ± 1.5	10.6 ± 1.7	9.7 ± 0.5	7.11 ± 0.11
12 min after injury									
H	7.37 ± 0.03	39 ± 4	48 ± 5	65 ± 13	343 ± 12	16.5 ± 0.7	5.8 ± 1.7	12.1 ± 0.5	7.12 ± 0.10
I	7.47 ± 0.04	32 ± 3	127 ± 8	96 ± 3	373 ± 14	17.3 ± 1.5	8.3 ± 1.5	10.7 ± 0.6	7.27 ± 0.05
I-H	7.29 ± 0.05	39 ± 3	48 ± 3	58 ± 7	326 ± 18	15.3 ± 1.2	11.8 ± 1.6	11.2 ± 1.0	7.05 ± 0.08
15 min after injury									
H	7.34 ± 0.06	38 ± 6	51 ± 7	65 ± 15	350 ± 15	16.4 ± 1.2	5.9 ± 1.9	11.8 ± 0.8	7.17 ± 0.11
I	7.40 ± 0.03	33 ± 1	134 ± 6	93 ± 6	373 ± 17	17.0 ± 1.0	6.9 ± 0.9	11.4 ± 0.7	7.25 ± 0.05
I-H	7.30 ± 0.05	38 ± 4	54 ± 5	57 ± 9	319 ± 16	14.5 ± 1.0	9.5 ± 1.2	11.6 ± 1.0	6.98 ± 0.14
21 min after injury									
H	7.32 ± 0.04	34 ± 3	130 ± 6	78 ± 12	330 ± 30	17.6 ± 1.1	6.4 ± 2.1	11.5 ± 0.4	7.17 ± 0.07
I	7.48 ± 0.18	31 ± 3	134 ± 2	98 ± 2	376 ± 12	17.1 ± 1.6	8.2 ± 1.1	10.5 ± 0.4	7.19 ± 0.07
I-H	7.28 ± 0.04	37 ± 3	131 ± 9	67 ± 12	313 ± 13	13.9 ± 1.6	6.9 ± 1.1	9.7 ± 1.2	7.01 ± 0.13
30 min after injury									
H	7.39 ± 0.02	31 ± 1	135 ± 6	95 ± 3	371 ± 23	17.4 ± 1.1	6.9 ± 0.9	11.7 ± 1.7	7.24 ± 0.07
I	7.43 ± 0.06	29 ± 1	128 ± 2	95 ± 6	376 ± 18	16.9 ± 1.6	7.8 ± 1.1	10.8 ± 0.5	7.20 ± 0.08
I-H	7.31 ± 0.03	30 ± 3	134 ± 9	87 ± 9	330 ± 11	17.6 ± 2.4	10.1 ± 1.4	11.7 ± 1.9	6.80 ± 0.21
45 min after injury									
H	7.42 ± 0.05	30 ± 4	131 ± 7	97 ± 3	353 ± 13	17.5 ± 0.5	6.8 ± 2.1	11.3 ± 1.0	7.14 ± 0.02
I	7.40 ± 0.05	29 ± 2	136 ± 1	100 ± 2	373 ± 14	17.7 ± 1.9	6.0 ± 0.9	10.9 ± 1.0	7.25 ± 0.13
I-H	7.38 ± 0.04	32 ± 1	136 ± 8	87 ± 6	370 ± 20	17.1 ± 1.1	8.6 ± 0.9	11.1 ± 1.2	6.90 ± 0.07
60 min after injury									
H	7.38 ± 0.08	30 ± 1	137 ± 6	97 ± 3	370 ± 21	17.5 ± 0.5	5.8 ± 2.0	10.2 ± .07	7.07 ± 0.04
I	7.54 ± 0.09	30 ± 1	140 ± 2	101 ± 2	393 ± 17	17.6 ± 0.7	6.1 ± 0.6	11.0 ± 0.7	7.18 ± 0.10
I-H	7.46 ± 0.07	30 ± 6	139 ± 9	95 ± 3	385 ± 35	17.6 ± 1.2	7.4 ± 0.7	11.3 ± 0.8	7.05 ± 0.11

[a] H, hypoxia alone; I, ischemia alone; H-I, hypoxia-ischemia.
[b] Percentage of total phosphate.

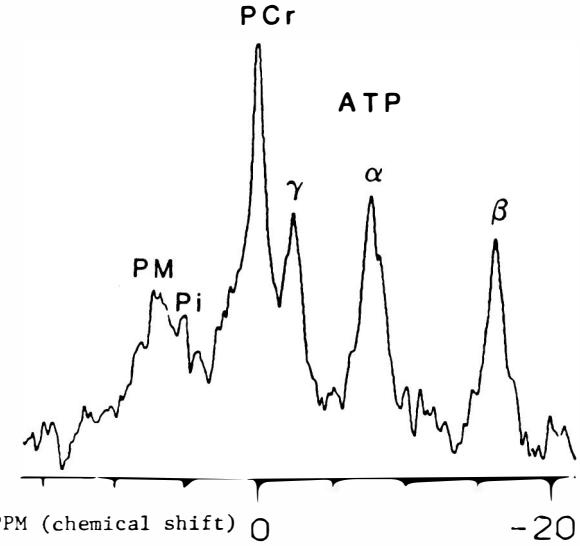

FIG. 1. Baseline "saturated" (interpulse delay 800 ms) ^{31}P spectrum. PM, phosphate monoester; Pi, inorganic phosphate; PCr, phosphocreatine; α, α-ATP; β, β-ATP; γ, γ-ATP.

ranged between 7.18 ± 0.05 and 7.33 ± 0.07. A significant change in pH values was noted in the ischemia and ischemia-hypoxia groups immediately (3 min) after insult. The lowest mean pH value in the ischemia-hypoxia group (6.80 ± 0.21) was found at 30 min after injury and at 3 min in the ischemia group (6.95 ± 0.12). These values returned toward baseline at 60 min in the ischemia-hypoxia group and at 9 min in the ischemia group. Changes in intracellular pH values are shown in Fig. 2.

Baseline PCr/P_{tot} values ranged between 17.3 ± 0.9 and 18.7 ± 0.9 (expressed as the percentage of total phosphate). A significant decrease in PCr was noted immediately after insult in the ischemia and ischemia-hypoxia groups. The lowest PCr value (13.9 ± 1.6) was found in the ischemia-hypoxia group 21 min after injury; a value of 15.6 ± 0.8 was found at 6 min in the ischemia group. PCr values returned toward baseline at 9 min in the ischemia group and at 30 min in the ischemia-hypoxia group. Changes in PCr are shown in Fig. 3.

Baseline mean values of Pi/P_{tot} ranged between 4.9 ± 0.7 and 5.1 ± 0.7 (expressed as the percentage of total phosphate). A significant increase in Pi values was noted within 3 min after insult in the ischemia-hypoxia group and within 6 min in the ischemia group. The highest Pi value of 10 ± 2.3 was observed at 6 min in the ischemia group, and the highest value of 11.8 ± 2.1 was found in the ischemia-hypoxia group at 3 min. Values of Pi that were significantly higher than baseline were observed up to 21 and 45 min in the ischemia and ischemia-hypoxia groups, respectively. Pi values returned toward baseline at the end of the MRS experiments (60 min). Changes in Pi values are shown in Fig. 4.

Intergroup analysis showed significantly higher Pi/P_{tot} values and lower pH and PCr/P_{tot} values in the ischemia-hypoxia group (Figs. 2–4). Changes in ATP values are shown in Fig. 5. No significant changes in pH, PCr, Pi, and ATP values were observed in the hypoxia group. Figure 6 shows baseline (I) and the 60 min spectra (III) of a typical rat of the ischemia group (A) and of the ischemia-hypoxia group (B). Spectra II A and B (Fig. 6) are examples of changes in phosphate metabolite peaks.

Histopathologic findings

No changes were found in the brains of the control and hypoxia alone groups. There was a 100% rate of infarction in the ischemic and ischemic-hypoxic groups. Changes seen in ischemia and ischemia-hypoxia groups were characterized by

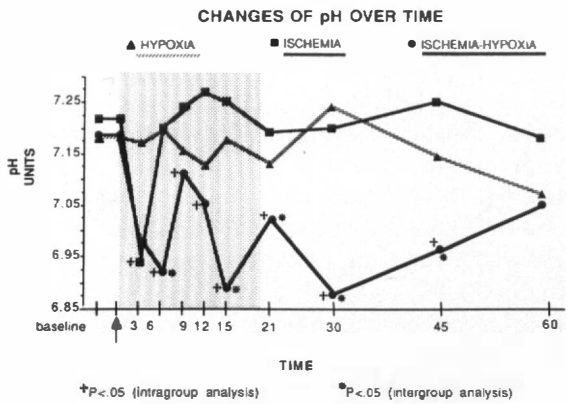

FIG. 2. Time course of intracellular pH values measured by ^{31}P MRS. Times represent the beginning of the spectra acquisition (3 min each). Arrow indicates induction of injury. Shaded area: duration of mild hypoxia (FiO_2 14%) in the hypoxia and ischemia-hypoxia groups. Data are shown as means.

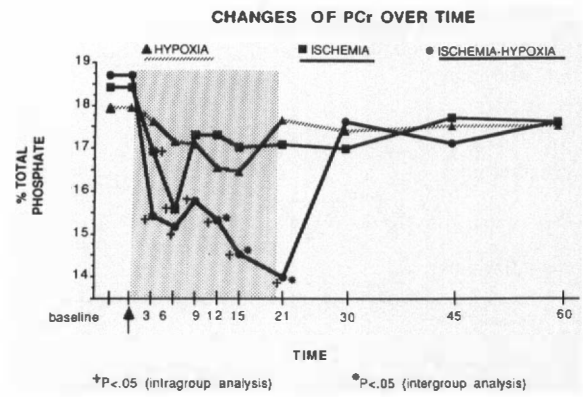

FIG. 3. Time course of PCr/P_{tot} ratios; values are expressed as the percentage of total phosphate. Times are the same as shown in Fig. 2. Arrow indicates induction of injury. Shaded area: duration of mild hypoxia (FiO_2 14%) in the hypoxia and ischemia-hypoxia groups. Data are shown as means.

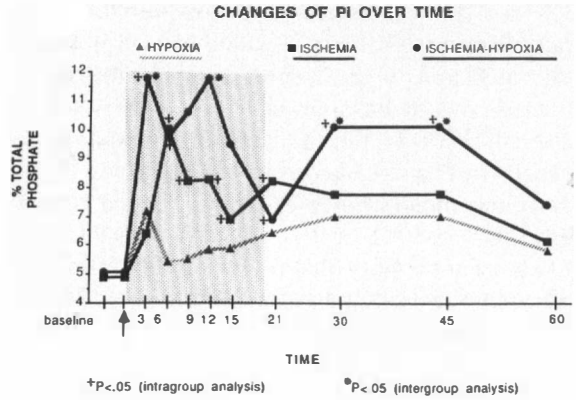

FIG. 4. Time course of Pi/P_{tot} ratios. Values are expressed as the percentage of total phosphate. Times are the same as shown in Fig. 2. Arrow indicates induction of injury. Shaded area: duration of mild hypoxia (FiO_2 14%) in the hypoxia and ischemia-hypoxia groups. Data are shown as means.

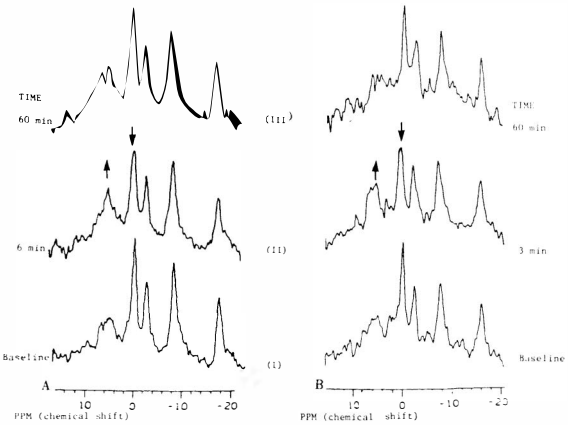

FIG. 6. Baseline (I) and last of the series (III) spectra of a typical rat in the ischemia group **(A)** and ischemia-hypoxia **(B)** group. Spectra II **A** and **B** represent the spectrum of each series that showed the greatest changes in phosphate metabolite peaks. Arrows indicate changes in phosphate peaks. Times are the same as shown in Fig. 2.

shrinkage and dark staining of the nuclei in the injured area, with vacuolization of the neuropil. Although no difference was noted between the two groups, there was a suggestion of more vacuolization and brain swelling in the ischemia-hypoxia group.

The size of infarction was not quantified because of the poor demarcation between ''normal'' and ischemic brain in rats killed as early as 1 h after injury. In rats killed 24 h after injury, infarcted tissue was a strip of superficial gray matter about 1.5 × 2.5 cm in the coronal section with the largest area of infarction, and extended approximately 2.5 cm in an anteroposterior direction, tapering at the anterior and posterior margins of the infarcted area.

DISCUSSION

Recent advances in MRS allow the noninvasive, sequential measurement of high energy cerebral metabolism in vivo (Chance et al., 1980; Hilberman et al., 1984; Gyulai et al., 1985), with simultaneous monitoring of other physiologic data (Naruse et al., 1984*a*). MRS has been used to measure the reduction of high energy phosphate metabolites in brain caused by acute hypovolemic-focal or global ischemia (Miyake, 1984; Horikawa et al., 1985).

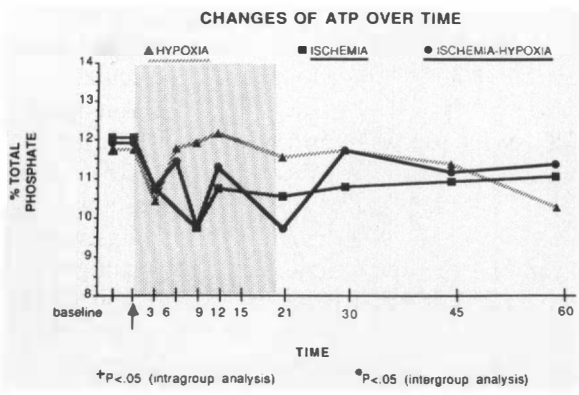

FIG. 5. Time course of ATP/P_{tot} ratios. Values are expressed as the percentage of total phosphate. Times are the same as shown in Fig. 2. Arrow indicates induction of injury. Shaded area: duration of mild hypoxia (FiO_2 14%) in the hypoxia and ischemia-hypoxia groups. Data are shown as means.

Phosphate metabolites may be studied in intact organs using surface coils that can be positioned directly over the tissue to be studied (Ackerman et al., 1980; James, 1984). Conditions in which the peak areas express the quantity of tissue metabolites can be achieved with a 12-s interpulse delay for ^{31}P—a ''fully-relaxed'' spectrum (Ackermann et al., 1980)—that requires 128 acquisitions collected in about 25 min. As we were interested in the early phases of ischemia, it was necessary to have a shorter period of data collection. We obtained ^{31}P ''saturated spectra'' with an interpulse delay of 800 μs and 224 acquisitions that could be collected in 3 min and gave satisfactory signal-to-noise ratios. Although absolute values of the concentration of phosphate metabolites in the tissue cannot be calculated with our data, changes in metabolites can be followed, and different metabolic states can be compared over time (Ackerman et al., 1980; Gadian, 1982).

One of the earliest changes seen after an acute hypoxic-ischemic insult is a progressive decline in PCr. Decreases in PCr and ATP occur in the ischemic brain and heart (Norwood et al., 1979; Thulborn et al., 1982; Gyulai et al., 1985); decreases of ATP occur in ischemic skeletal muscle only after drastic depletion of PCr (Ackerman et al., 1980). The isoenzymes of creatine kinase (Thulborn et al.,

1982) and the mitochondrial compartmentalization (Norwood et al., 1979) are different in the two tissues, which may explain differences in the time course of changes in levels of these metabolites. Hilberman et al. (1984) reported that ATP decreased only after PCr decreased to about 45% of baseline levels. Litt et al. (1986) found no change in ATP with decreases in PCr of about 25%. In agreement with these authors, we found that significant changes in PCr (approximately 30% of baseline values) did not cause a significant change in ATP levels.

We can only speculate on the reasons that changes in ATP were not more dramatic. Although reduction in ATP paralleled changes in Pi and PCr in all groups (minimal changes with hypoxia alone, larger reductions in the ischemic and ischemic-hypoxic group) they were not significantly reduced. It is possible that statistically significant differences might have been seen in larger groups of rats, but there are other possible explanations. The volumes of infarcted tissue were smaller than the volume of tissue from which phosphate spectra were recorded with the surface coil placed directly over the area of infarction; undoubtedly, phosphate in normal tissue contributed to the high energy phosphate peaks. The use of smaller coils leads to lower signal-to-noise ratios, and phosphate peaks become difficult to quantify. This problem is exacerbated for measurement of the Pi peak, which overlaps peaks for phosphate monoesters and for γ-ATP, the peak of which overlaps PCr. Even though the 5.6 tesla magnet used in these studies provides unusually good spectra, precise quantification of the high energy phosphates in a small volume of brain poses substantial problems. Nonetheless, the effort seems to be justified, as there is currently no other way to follow alterations in brain phosphates sequentially in live animals or in humans.

Intracellular pH can be determinated noninvasively by MRS using the chemical shift of Pi relative to PCr (James, 1984). Mean baseline pH values found in this study were between 7.18 ± 0.05 and 7.33 ± 0.07, which are similar to values found by Thulborn et al. (1982) in the gerbil (pH 7.2) and are consistent with values found in buffered-perfused rat brain (pH 7.2) by Norwood et al. (1979). The lowest pH values found at 12 min and 3 min after insult in the ischemia-hypoxia and ischemia groups, respectively, reflect intracellular acidosis (Hilberman et al., 1984; Smith et al., 1986).

Characteristic changes in neurons observed with light microscopy after a critical reduction in cerebral blood flow was described by Spielmeyer (1922) as "ischemic cell change" that consisted of shrinkage and hyperchromasia of the cell body and development of a triangular nucleus. These changes lead eventually to cell death and disappearance. The earliest phase of ischemic cellular change in brains exposed to nitrogen for 30 min and immediately fixed was described by Brown and Brierley (1968) as "microvacuolization." These findings were confirmed by several authors in other models of hypoxic-ischemic insults (Brierley et al., 1970; McGee-Russel et al., 1970; Garcia et al., 1983). Recent evidence suggests that it is possible to detect postischemic neuronal changes within 10 min of insult using immunohistochemical evaluation of the cytoskeleton proteins (Yanagihara et al., 1985).

Our histopathologic findings based on light microscopy with H & E staining agree well with reported observations. The deleterious effects of hypoxia (P_aO_2 35–40 mm Hg) on neurologic outcome and cerebral tissue in experimental head injury have been reported (Nelson et al., 1982; Ishige et al., 1987); Blennow et al. (1979) did not find these effects in experimental epilepsy. To our knowledge, there have been no reports of the effects of mild hypoxia (P_aO_2 of ca. 48 mm Hg) on cerebral ischemia. Our findings that there is a greater vacuolization of the neuropil in the ischemia-hypoxia group than in the ischemic group suggests that hypoxia adversely affects the outcome of cerebral ischemia. It is not known whether the adverse effects of hypoxia on tissue cause a worse neurologic outcome and/or cause more histopathologic damage in surviving animals; this question should be investigated further.

Changes in cerebral metabolism in vivo have been documented after hypoxic or ischemic-hypovolemic insult using MRS (Thulborn et al., 1982; Miyake, 1984; Naruse et al., 1984*b;* Horikawa et al., 1985; Litt et al., 1985). If the noxious stimulus is discontinued within 30–60 min of the initial insult, ^{31}P spectra "recover." Despite a paucity of data on focal cerebral ischemia, it was reported recently that ^{31}P spectra "recovered" after circulation in a model of focal cerebral ischemia (Welch, 1986). Our results show a "recovery"—a return toward baseline values—in ^{31}P spectra despite a permanent ischemic insult and a histologically documented area of infarction. These findings were unexpected. The existence of steep oxygen gradients in tissues has been reported (Stuart and Chance, 1974; Tamura et al., 1984) and has been proposed as the source of hetereogeneity in the hypoxic-ischemic brain (Chance et al., 1986). Therefore, metabolic heterogeneity, differential saturation effects that occur during the recording of spectra (James, 1984), and difficulties inherent in the use of the sur-

face coil may influence the results of ^{31}P MRS. In addition, clearance of Pi from infarcted brain into the venous circulation is known to occur in humans (Meyer et al., 1975). Spectra recorded after washout of Pi may return toward normal, even though brain infarction is present. As in many experimental models, differences in species studied and specific models used warrant caution in the generalization of results for different experimental conditions. Nonetheless, our results suggest that great caution is appropriate in the interpretation of "recovery" of ^{31}P MRS.

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