Magnetic Resonance Imaging and ^{31}P Magnetic Resonance Spectroscopy Study of the Effect of Temperature on Ischemic Brain Injury

Garnette R. Sutherland, Howard Lesiuk, Paul Hazendonk, James Peeling, Richard Buist, Piotr Kozlowski, Andrzej Jazinski and John K. Saunders

ABSTRACT: Transient forebrain ischemia was induced in rats whose brain temperature was 31, 33, 35, 38, or 40°C. The development of regional injury was followed using magnetic resonance (MR) imaging, with the ultimate extent of neuronal injury quantified histopathologically. Animals in the hypothermic groups showed minimal changes in MR images over 4 days; normothermic animals showed intensity enhancement attributed to progressive edema developing in the striatum and, later, in the hippocampus. Ischemia at 40°C resulted in widespread edema formation by 1 day post-ischemia; animals in this group did not survive beyond 30 hours. Histopathological analysis at 4 days (1 day for the hyperthermic group) post-ischemia showed that neuronal damage in the normothermic group was confined to the hippocampus and striatum. Minimal damage was found in the hypothermic groups; damage in the hyperthermic group was severe throughout the forebrain. There were no differences in the pre-ischemia ^{31}P MR spectra for the different groups. During ischemia, the increase in intensity of the Pi peak and the fall in tissue pH increased with temperature in the order hypothermic < normothermic < hyperthermic group of animals. Post-ischemia energy recovery was similar in all groups, while pH recovered more rapidly in hypothermic animals.

RÉSUMÉ: Étude de l'effet de la température sur la lésion cérébrale ischémique au moyen de l'imagerie par résonance magnétique et de la spectroscopie par résonance magnétique au P^{31}. Une ischémie transitoire du cerveau antérieur a été induite chez des rats dont la température cérébrale était de 31, 33, 35, 38 ou 40°C. L'évolution de lésions régionales a été suivie par imagerie par résonance magnétique (MRI) et l'étendue finale des lésions neuronales a été quantifiée par histopathologie. Chez les animaux des groupes hypothermiques, les changements d'images MRI étaient minimes sur 4 jours; les animaux normothermiques avaient une augmentation de l'intensité du signal attribuée au développement d'un oedeme progressif dans le striatum et, plus tard, dans l'hippocampe. Une ischémie à 40°C provoquait la formation d'un oedeme étendu à 1 jour post-ischémie; les animaux de ce groupe n'ont pas survécu plus de 30 heures. L'analyse histopathologique au 4e jour post-ischémie (à 1 jour pour le groupe hyperthermique) a montré que le dommage neuronal chez le groupe normothermique était limité à l'hippocampe et au striatum. Des dommages minimes ont été constatés chez les groupes hypothermiques; les dommages chez le groupe hyperthermique étaient sévères dans tout le cerveau antérieur. Il n'y avait pas de différence entre les groupes dans les spectres au P^{31} obtenus par résonance magnétique avant l'ischémie. Pendant l'ischémie, l'augmentation de l'intensité du pic Pi et la chute pH tissulaire avec la température ont augmenté, l'ordre des groupes d'animaux étant hypothermiques < normothermique < hyperthermique. La récupération énergétique post-ischémie a été semblable dans tous les groupes, alors que le pH s'est rétabli plus rapidement chez les animaux hypothermiques.

Can. J. Neurol. Sci. 1992; 19: 317-325

Patients with neurosurgical disorders, in particular those with intra-cranial hemorrhage, are often mildly hyperthermic. Furthermore, secondary to the effects of general anesthetics, mild hypothermia often occurs during intracranial procedures, and post-operatively patients can develop mild hyperthermia in response to infection or surgical insult. As many of these disorders and their surgical treatment are accompanied by ischemia (through compression associated with neoplasms, hemorrhage, or retractors, edema, vascular occlusion accompanying vasospasm, temporary vascular clips, or thromboembolism) the effects of mild fluctuations in temperature on ischemic brain injury must be considered. While profound hypothermia (brain temperature of ≤ 30°C) was used in the past to ameliorate neuronal injury during neurosurgical procedures,[1-4] the effects of mild temperature variation (± 4°C or less) have not been thoroughly documented in the clinical setting. However, using animal models of

From the Departments of Surgery (Neurosurgery), Pharmacology, and Radiology, The University of Manitoba; Department of Chemistry, University of Winnipeg, Winnipeg; and Division of Biological Sciences, National Research Council of Canada, Ottawa

Received October 11, 1991. Accepted in final form March 9, 1992

Reprint requests to: Dr. James Peeling, Department of Pharmacology, The University of Manitoba, 770 Bannatyne Avenue, Winnipeg, Manitoba, Canada R3E 0W3

cerebral ischemia, protection against neuronal injury with mild hypothermia and accentuation of damage with mild hyperthermia has been demonstrated.[5-11]

As the mechanisms of ischemic-induced neuronal injury itself are incompletely understood, the origin of the observed temperature effects remains somewhat speculative. However, the rates of enzyme-catalyzed reactions are markedly temperature dependent,[12] so that during profound hypothermia in dogs the cerebral metabolic rate decreases to 25-35% of normal.[13,14] Changes in the rates of metabolic processes with temperature during and following ischemia may therefore be important. During ischemia, metabolic activity rapidly depletes the available reserves of adenosine triphosphate (ATP) and phosphocreatine (PCr), leading to energy failure, and anaerobic metabolism of residual tissue glucose leads to lactic acidosis.[15] Following reperfusion, the energy state, acidosis and metabolic function may recover.[15] Subsequently, developing neuronal injury in animal models of ischemia is accompanied by edema formation in the brain regions where damage occurs.[16-19] Clinically, ischemic brain injury is often similarly accompanied by progressively developing edema, which may be associated with clinical deterioration.[20-21] The effect of brain temperature on the rate and extent of ischemic energy failure and acidosis, on the subsequent recovery following reperfusion, and on the development of edema following an ischemic event, have not been examined. Studies of these effects may improve the understanding of the mechanistic aspects of the temperature dependence of ischemic brain injury.

In the present study the effects of mild hypothermia and hyperthermia on short-duration forebrain ischemia in the rat have been examined. *In vivo* ^{31}P magnetic resonance (MR) spectroscopy has been used to follow ischemic energy failure and tissue acidosis, and their recovery following reperfusion. MR imaging has been used to follow the time course of regional post-ischemic edema. The results are correlated with histopathologically assessed tissue injury.

Methods and Materials

Measurement of Brain Temperature

Twelve male Sprague-Dawley rats, 350-450g, were used to evaluate the temperature measurement and maintenance procedures. Anesthetic management, surgical preparation, induction of ischemia, and post-ischemic care are described below. In each rat a thermocouple probe (Physitemp Inst. Inc. RET-1) was inserted into the descending colon through the rectum, and a second probe (Physitemp Inst. Inc. IT-18)— was placed through the tympanic membrane into the middle ear. A third thermocouple probe .07mm in diameter (Physitemp Inst. Inc. MT-29/1) was inserted through a right frontal burr hole into the brain to a depth of 4 mm. This electrode was fixed in position with n-butyl-2-cyanoacrylate, and the scalp was closed around the thermocouple lead. Pre-ischemia, ischemia, and post ischemia temperature readings were obtained from each thermocouple at 1 minute intervals in groups of 4 animals. In one group, no attempt was made to maintain head temperature, while the rectal temperature was maintained as close as possible to 38°C. In the remaining 2 groups, the middle ear temperature was maintained as close as possible to 35° or 40°C. Following ischemia the animals in the 40°C group were cooled to 39°C. The animal's temperature in each case was adjusted and regulated by heating or cooling with water from a heating/cooling circulating water bath (Lauda RMS-6) passed through a water blanket placed under the rodent's head and body. Heating was supplemented by a rheostatically controlled heat lamp, and cooling was supplemented by air blown over the animal and by the application of external ice packs as needed.

Induction of Reversible Forebrain Ischemia

All experiments used male Sprague-Dawley rats weighing 350-450 g. Each animal was pre-treated with atropine (0.5 mg/kg) to reduce oropharyngeal secretions and vagal response to intubation, and then anesthetized with sodium pentobarbital (60 mg/kg).[18,19,22,23] Each rat was intubated and mechanically ventilated. Continuous temperature monitoring was carried out with the tympanic thermocouple probe as described above. The animal's temperature was adjusted to and maintained at a nominal tympanic probe temperature of either 31° (n = 7), 33° (n = 15), 35° (n = 9), 38° (n = 13), or 40°C (n = 14), using the temperature control procedures described above.

For all animals the desired temperature was stabilized prior to the induction of ischemia and maintained until 40 min. post-ischemia, except for those in the 40°C group. These animals did not survive recirculation by more than a few hours if the temperature was maintained at 40°C following the ischemia. Consequently, for the 40°C group the protocol was modified to include lowering the temperature to 38°C immediately post ischemia by cooling the animal's extracorporeal blood (which had been withdrawn to induce systemic hypotension) prior to blood reinfusion in addition to the application of external ice packs and cooled water in the water blanket. For rats being studied by ^{31}P MR spectroscopy, the temporalis muscles were resected to decrease signal from tissue other than brain, and a 2-turn eliptical surface coil (1.2 × 1.0 cm) was sutured under the scalp.

In each rat, the tail artery was catheterized to permit blood pressure monitoring, induction of systemic hypotension, and monitoring of blood gases and serum chemical composition. Both carotid arteries were exposed through a neck incision. After 20 minutes of stabilization, forebrain ischemia was induced through bilateral carotid occlusion coincident with a reduction in systemic blood pressure to a mean of 50 mm/Hg through aspiration of blood into a heparinized syringe. After 10 minutes, blood flow through the carotid arteries was restored and the aspirated blood was reinfused. Blood gas analysis and hematocrit determinations were obtained both prior to and following the ischemic insult. For animals undergoing imaging studies, ventilatory support was continued until the animal was breathing well and moving its extremities. For animals being examined by ^{31}P MR spectroscopy, ventilatory support was continued for the duration of the study.

^{31}P MR Spectroscopy

All MR examinations were performed on a Bruker Biospec 4.7/30 spectrometer. ^{31}P MR spectroscopy studies were carried out on animals with brain temperatures of 33°, 38°, and 40°C (5 rats in each group). Four ^{31}P MR spectra were obtained prior to ischemia, 8 during ischemia, and 36 spectra were obtained following reperfusion. Each free induction decay (FID) was collected with 32 transients into 4 K data points in 1 minute. Each

https://doi.org/10.1017/S0317167100041937 Published online by Cambridge University Press

FID was multiplied with a 12 point trapezoidal function to decrease the contribution of the broad spectral background peak from phosphates in the skull, and by a 30 Hz line broadening funtion to improve signal to noise, before Fourier transformation to give the spectrum.

Peak heights, measured relative to the pre-ischemia PCr for each rat, were used to follow changes in the levels of inorganic phosphate (Pi), ATP (using the β-ATP resonance), and PCr. Tissue pH was measured from the difference in the chemical shifts of the Pi and PCr resonances.[24]

MR Imaging

Animals in the normothermic group (ischemia at 38°C, n = 8) were examined by MR imaging on days 1, 2, and 4 post-ischemia to confirm the previously established temporal profile of ischemic-induced MR imaging changes.[18,19] Based on the results of these studies, animals in the hypothermic groups (ischemia at 31, n = 7, 33, n = 10, or 35°C, n = 9) were imaged on day 2, when imaging changes in the normothermic group were clearly apparent. In addition, a number of the animals were also imaged on days 1 and/or 4 post-ischemia to monitor potential changes in the temporal profile. In the case of the 40°C (n = 9) animals, the severe nature of the resulting ischemic injury caused high mortality between 24 and 30 hours post ischemia so that images could be obtained only on day 1 post-ischemia.

In the spectrometer the rats were immobilized with 1% isoflurane in 30% O_2/70% room air administered via a nose cone. Normal body temperature was maintained by circulating 38°C air over the animal and supporting the animal on a temperature regulated water blanket. All imaging examinations were performed using a modified saddle coil 3.5 cm in diameter and 4 cm long. The animal's head was fixed in position using an incisor bar, with the brain centered in the coil. A scout projection image taken vertically through the head was used to select consistent coronal image locations, one through the striatum and another through the hippocampus at the level of the thalamus. Images were acquired using a multi-slice (2 locations) dual echo sequence (TE = 22 and 66 msec, TR = 1365 ms, 8 accumulations, slice thickness 1.5 mm). Each image was acquired using 256 × 256 data points with a field of view of 4.0 × 4.0 cm, giving a pixel resolution of about 150 μm.

Images obtained from each rat were examined by an investigator unaware of the treatment group. Regional (striatum, hippocampus, neocortex, thalamus) differences compared to images from non-ischemic rats were graded using the strongly T_2-weighted image, with 0 = not distinguishable from normal, 1 = mild to moderate increase in intensity, and 2 = marked increase in image intensity. MR images which were judged by the examiner to be sub-optimal (for instance due to positioning or motion artifact) were not graded.

Histopathology

Following the final imaging study for animals in the 40°C and 38°C groups and on day 5 post-ischemia for the hypothermic groups, each rat was perfusion-fixed with one litre of 10% buffered formaldehyde (pH 7.25). After fixation, the brain was placed in the same fixative for two weeks prior to sectioning. The brains were cut coronally into 1.5 mm slices, dehydrated in graded concentrations of ethanol, and embedded in paraffin. Serial sections 8 μm thick were stained with hematoxylin and eosin. All sections were examined to determine the qualitative and topographic extent of brain damage. For quantitation of ischemic neuronal injury, standardized sections of the cerebral cortex, hippocampus, and striatum were used. Pre-determined regions of the hippocampal and frontal sections were photographed and ischemic neuronal injury was quantitatively determined by direct visual counting of all neurons.[19,23] The frequency of ischemic neurons was calculated by dividing the number of acidophilic and/or pyknotic neurons by the total number of neurons. Damage within the dorsal lateral striatum was graded according to the degree of neuronal necrosis, with fewer than 10% necrotic neurons = 1; 10-50% necrotic neurons = 2; 50-100% necrotic neurons = 3. The examiner was unaware of the identity of the sections examined.

Statistical Analysis

Comparisons of the physiological and histological data were carried out with an analysis of variance and Scheffé's intergroup comparison test. Repeated-measures least-squares analyses were applied to the pre-ischemia, ischemia and post-ischemia ^{31}P MR data for levels of Pi, ATP, and PCr, and for tissue pH.

Results

Control of Brain Temperature

Comparison of Brain, Tympanic, and Rectal Temperatures

Rectal, tympanic, and brain temperature measurements for the 35, 38, and 40°C groups are presented in Figure 1. For the 38°C group in which no attempt was made to maintain tympanic temperature, a progressive decrease in both tympanic and brain temperatures occurred during the 10 minutes of ischemia, with the brain and tympanic temperatures generally agreeing to within ± 0.2°C (maximum difference 0.8°C). The rectal temperature remained constant, so that by the end of 10 minutes of ischemia the brain temperature was as much as 2.6°C lower than the rectal temperature. On reperfusion, the rectal temperature fell significantly as non-thermostated blood was reinfused. The brain and tympanic temperatures remained constant through 5 minutes of reperfusion. In rats in which the tympanic temperature was maintained to within ± 0.3°C of 40°C or 35°C, the rectal temperature was consistently higher (as much as 1.5°C) than the tympanic or brain temperatures during ischemia. The brain and tympanic temperatures, however, agreed closely, generally differing by less than 0.2°C (maximum difference 0.6°C). In these tightly controlled rats the tympanic, brain, and rectal temperatures became similar on reperfusion following ischemia.

Experimental Temperature Groups

Figure 2 shows the tympanic membrane temperature before, during, and following ischemia in groups of temperature-controlled animals. Throughout the experiment, no significant temperature variations occurred except for the 40°C group in which the temperature was intentionally reduced post-ischemia. For an individual animal, the variation in tympanic temperature through the course of the experiment was 0.39 ± 0.18°C, with the maximum variation being 0.8°C.

General Observations

A number of qualitative observations made during the course of the experiment suggest that ischemia induced at lower

https://doi.org/10.1017/S0317167100041937 Published online by Cambridge University Press

temperatures produced less injury than ischemia occurring at higher temperatures. At higher temperatures greater peri-ischemic blood pressure lability was observed. The hypothermic animals recovered from anaesthesia more rapidly than the normothermic animals, and the rats whose temperature was maintained at 40°C required prolonged ventilatory support followed by close observation with frequent oral suctioning to prevent aspiration. After overnight recovery, the hypothermic rats resumed superficially normal feeding, drinking, and general activity whereas the 38°C animals were lethargic. The 40°C animals fluctuated between profound lethargy and pronounced irritability, culminating in generalized seizure activity about 24 hours post-ischemia, and death 24-30 hours post-ischemia, usually after repeated seizures. A correlation was noted between peri-ischemic temperature and mortality, most dramatically manifest in a cohort of 41°C animals, a group which was initially planned for this study, but which had to be abandoned when 5 of 5 animals died within 1-2 hours of the ischemic insult.

Physiological Variables

Mean blood pressure increased slightly following the ischemic insult from 139 ± 3 to 152 ± 3 mm Hg ($p < 0.001$). In the 38 and 40°C groups, pre-ischemic blood pressures were higher (and consequently the blood pressure increase post-ischemia was less pronounced) than in the hypothermic animals ($p < 0.05$). Blood gas values ($PaCO_2$ and PaO_2) both pre- and post-ischemia were within normal limits for all experimental groups. The ischemic insult produced a mild systemic acidosis (pH fell from 7.38 ± 0.01 pre-ischemia to 7.31 ± 0.01 post-ischemia; $p < 0.001$) with no significant difference in the degree of acidosis between the different temperature groups.

^{31}P MR Spectroscopy

This forebrain ischemia model has previously been shown to produce rapid energy failure, evidenced by a decrease in the intensity of peaks due to high energy phosphate compounds and a rise in the inorganic phosphate peak in the *in vivo* ^{31}P magnetic resonance spectrum.[18] This is shown in the spectra in Figure 3 obtained from a typical normothermic rat before, during, and following cerebral ischemia. Qualitatively similar behavior was observed for all other animals studied. Analysis of the spectra did not identify any differences before ischemia in the relative levels of Pi, PCr, and ATP, or in the cerebral pH, between the hyperthermic (40°C), normothermic (38°C), or hypothermic (33°C) groups of animals. During ischemia significant differences were not observed between the groups for changes in ATP or PCr, although the adverse signal-to-noise level may have prevented the observation of small differences in the intensities of the small ATP and PCr peaks. However, the relatively intense Pi peak, originating from the hydrolysis product of both ATP and PCr, had sufficient signal-to-noise during ischemia to show that at higher temperatures Pi increased more rapidly (40° > 37°, $p = 0.081$; 37° > 33°, $p = 0.018$; 40° > 33°, $p < 0.0001$) and to a greater extent (40° > 37° or 33°, $p < 0.001$) as shown in Figure 4. During ischemia the fall in pH occurred more slowly in the hypothermic group compared to the normothermic ($p = 0.135$) or the hyperthemic ($p = 0.0025$) groups, and was somewhat less severe in the hypothermic animals (Figure 4).

Following reperfusion the PCr, ATP, and Pi peaks in the ^{31}P MR spectra recovered within the first 5 minutes to pre-ischemia

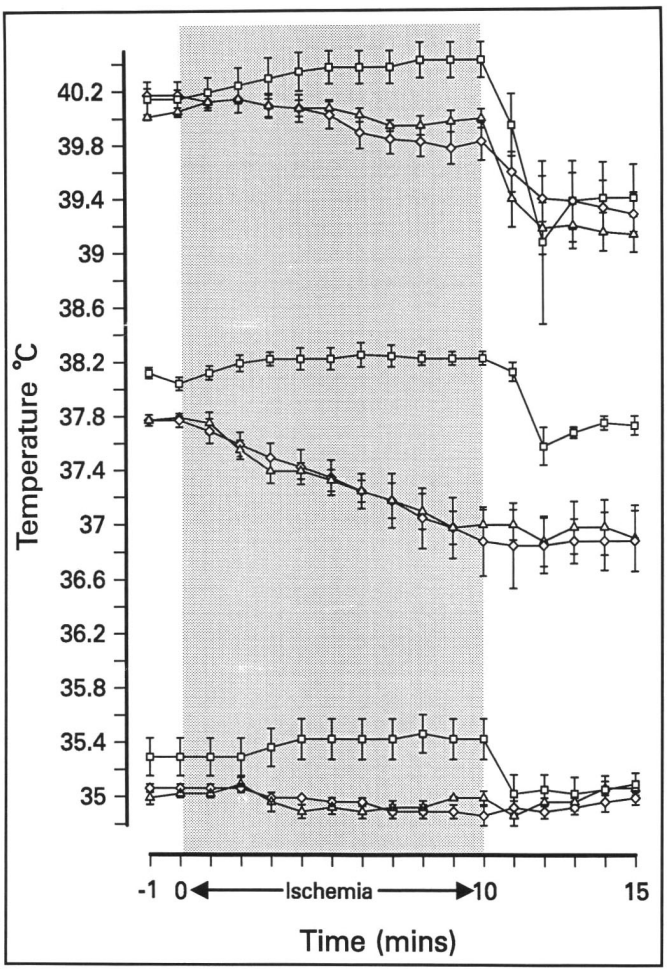

Figure 1 — Rectal ( ), tympanic (Δ·), and brain (◊) thermocouple temperatures recorded before, during, and following transient forebrain ischemia. Animal temperature was controlled to maintain a constant rectal temperature (38°C group) or tympanic temperature (35°C, 40°C groups). The temperature of the 40°C animals was intentionally decreased post-ischemia. Each point is the mean ± SEM for measurements on 4 rats. The shaded region represents the interval of cerebral ischemia.

levels, with no differences between groups. Restoration of pH to the pre-ischemia value was faster in the hypothermic group than in the normothermic ($p = 0.17$) or hyperthermic ($p = 0.006$) animals, and was complete by 20 minutes post-ischemia in all cases, as shown in Figure 4.

MR Imaging

The hypothermic (31°, 33°, 35°C) groups of animals showed no, or minimal, changes in the MR images over 4 days post-ischemia (Table 1, Figure 5). In the 38° animals, progressive enhanced intensity was evident in the T_2-weighted images, involving primarily the striatum on day 1 post-ischemia and including both the striatum and hippocampus on days 2 and 4. Ischemia at 40°C resulted in widespread intensity enhancement by 1 day post-ischemia.

Qualitative Histopathological Analysis

In rats given an ischemic insult at 38°C neuronal injury was primarily confined to the hippocampal CA1 and CA2 sectors, to

https://doi.org/10.1017/S0317167100041937 Published online by Cambridge University Press

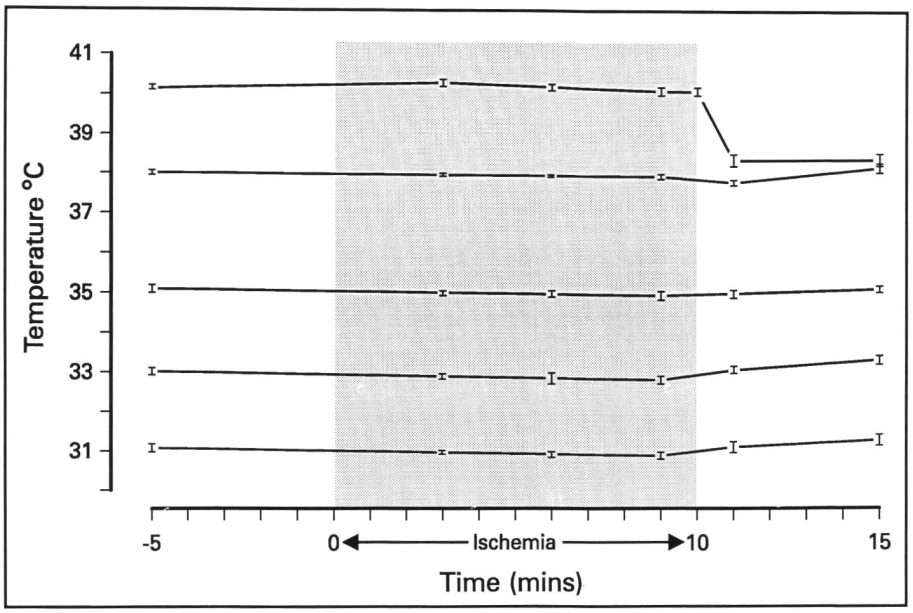

Figure 2 — Tympanic membrane temperatures of the experimental groups of rats (mean ± SEM) given an ischemic insult at controlled temperatures. The shaded region represents the interval of cerebral ischemia.

Table 1. Graded Regional MR Image Intensity at Successive Days Post-Ischemia for Individual Rats

	31°C	33°C	35°C	38°C	40°C
Day 1					
Striatum	0000	000	00000	100111	11112212
Hippocampus	0000	000	00000	020000	10111012
Thalamus	0000	000	00000	000000	00110002
Frontal Cortex	0000	000	00000	000000	10200012
Day 2					
Striatum	000000	000000	0000000	10101101	
Hippocampus	001000	100000	0000000	11102211	
Thalamus	000000	000000	0000000	00000000	
Frontal Cortex	000000	000000	0000000	10000000	
Day 4					
Striatum	000000	0000	000	0011	
Hippocampus	000000	0001	000	0211	
Thalamus	000000	0000	000	0000	
Frontal Cortex	000000	0000	000	0000	

Grade: 0 = same as non-ischemic.
1 = mild/moderate intensity increase.
2 = marked intensity increase.

a lesser extent the dorsal lateral striatum, and in four rats, to mild changes in the deep cortical layers (layers III through VI). Neocortical injury was essentially confined to the watershed territory between the middle and anterior cerebral arteries, tapering posteriorly as the territory supplied by the posterior cerebral artery was approached. This distribution of injury is the same as that observed in this forebrain ischemia model when volatile anesthetics were used and discontinued 30 minutes prior to ischemia,[25] suggesting that anesthetic management has minimal effect on the extent of neuronal damage. The same regional distribution of neuronal injury, but much less severe, was observed in rats subjected to ischemia at 31°, 33°, or 35°C. In these groups, 2 of the 31°, 6 of the 33°, and 4 of the 35° animals had no apparent neuronal injury. Neuronal damage was more severe and more widespread in the hyperthermic (40°C) animals, involving all layers of the neocortex, all hippocampal sectors including the dentate gyrus, the entire striatum, and portions of the thalamus. Neuronal injury in the cerebellum was confined to 7 rats in which mild injury was evident in Purkinje cells of the superior vermis.

The neocortex, hippocampus, striatum, and portions of the thalamus of the 40°C rats had a spongy appearance, while in the 38°C animals such changes were milder and were confined to the hippocampal CA1 and CA2 sectors and the dorsal lateral striatum. This spongy appearance was not apparent in any of the animals of the hypothermic groups.

https://doi.org/10.1017/S0317167100041937 Published online by Cambridge University Press

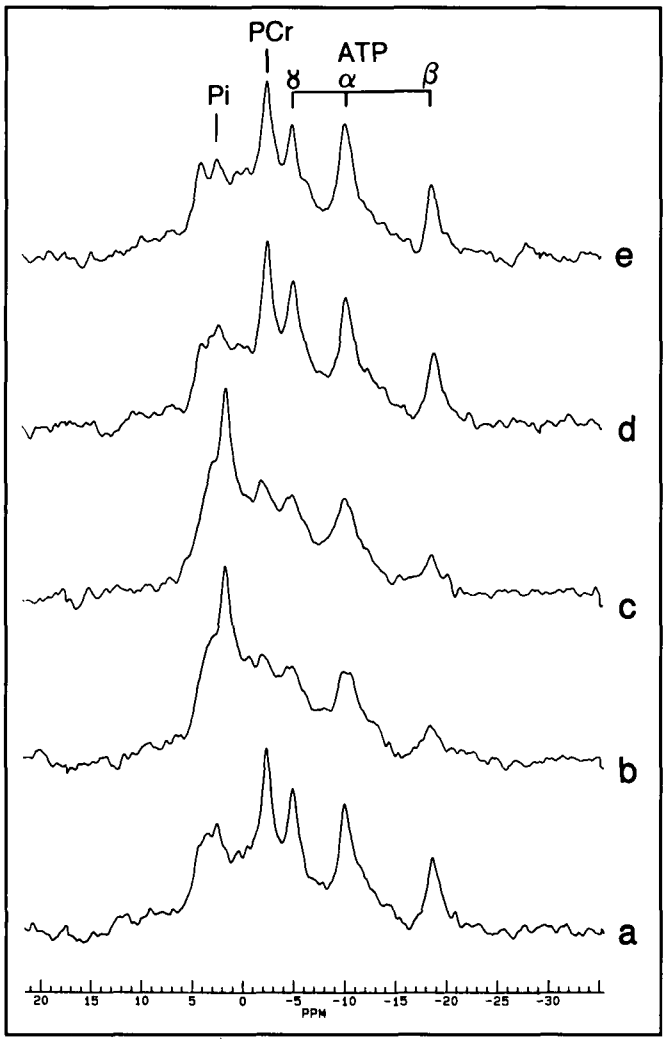

Figure 3 — In vivo ^{31}P *MR spectra of the brain of a normothermic rat (a) before ischemia, (b,c) at successive times during ischemia, and (d,e) following reperfusion. Each spectrum is the sum of four 1-minute spectra.*

Quantitative Histopathological Analysis

Quantitative histopathological data are presented in Figure 6. In all regions ischemic neuronal injury was similar in the three hypothermic groups with no significiant intergroup differences. In hippocampal sectors CA1 and CA2, ischemic neuronal injury was equally severe in both the 38 and 40°C groups and significantly greater than in the hypothermic groups. In the CA3 sector ischemic neuronal injury was greater in the 38°C group compared to hypothermic animals and greater still in the 40°C group. In the CA4 sector the degree of injury was not significantly different between the normothermic and hypothermic groups, while the 40°C group showed more injury than the other groups.

Within the frontal cortex ischemic neuronal damage was not significantly different between the 38°C and hypothermic groups. Injury was extensive in hyperthermic animals and significantly greater than in the other groups. Striatal injury was similar to that in the CA3 hippocampal sector, with greater injury in the 38°C group compared to hypothermic groups, and the most severe injury occurring in the 40°C group.

Discussion

For small temperature changes such as those used in the present study, the rates of enzyme-catalyzed reactions increased exponentially with temperature. However, in the absence of ischemia, the ^{31}P MR spectroscopy results show that there is no change in the cerebral energy state or pH with changes in temperature, implying close coupling between cerebral blood flow and metabolism at all temperatures.

During ischemia, the uncoupling of cerebral blood flow and metabolism results in cerebral energy failure, and the associated dissipative ion fluxes result in the intracellular accumulation of calcium.[26] This activates various lipolytic, proteolytic, and nucleolytic enzyme systems[26-29] which if severe and prolonged lead to irreversible cell injury. The more rapid and extensive energy failure demonstrated in the ^{31}P MR spectroscopy results together with increased rates of the resulting catabolic processes at higher temperature may account for the more extensive tissue injury evident in the histopathological data and in the more marked edema evident in the MR images of the hyperthermic animals. Similarly, the slower energy failure apparent in the ^{31}P MR

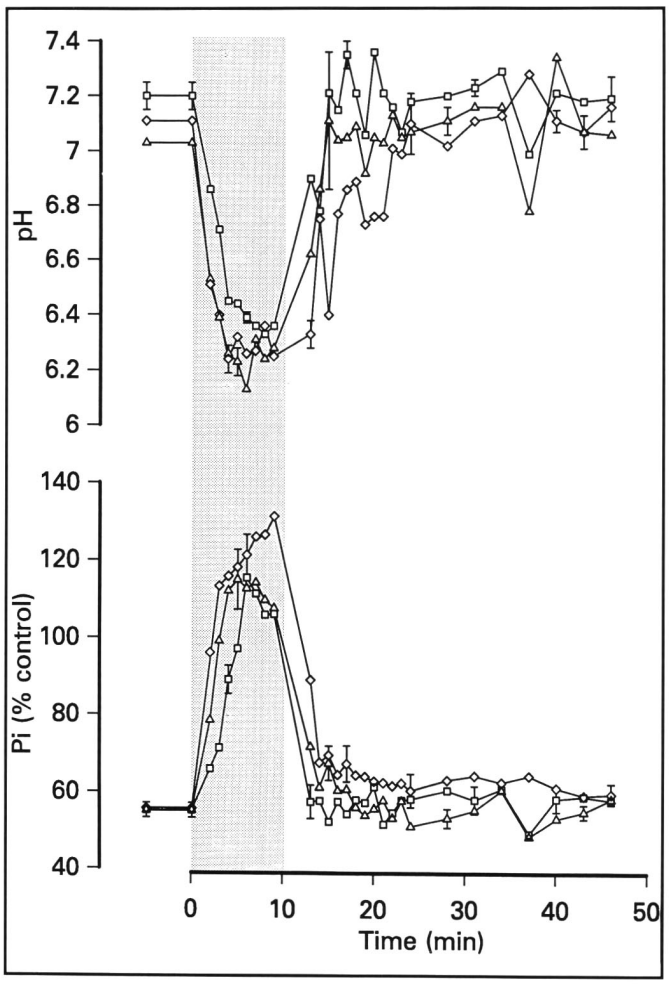

Figure 4 — Cerebral pH (above) and intensity of the Pi peak as percent of pre-ischemia PCr peak intensity (below) determined from ^{31}P MR spectra before, during (shaded), and following ischemia for the hyperthermic (◊), normothermic (Δ), and hypothermic ( ) groups of rats. Error bars represent typical SEM for several points.

https://doi.org/10.1017/S0317167100041937 Published online by Cambridge University Press

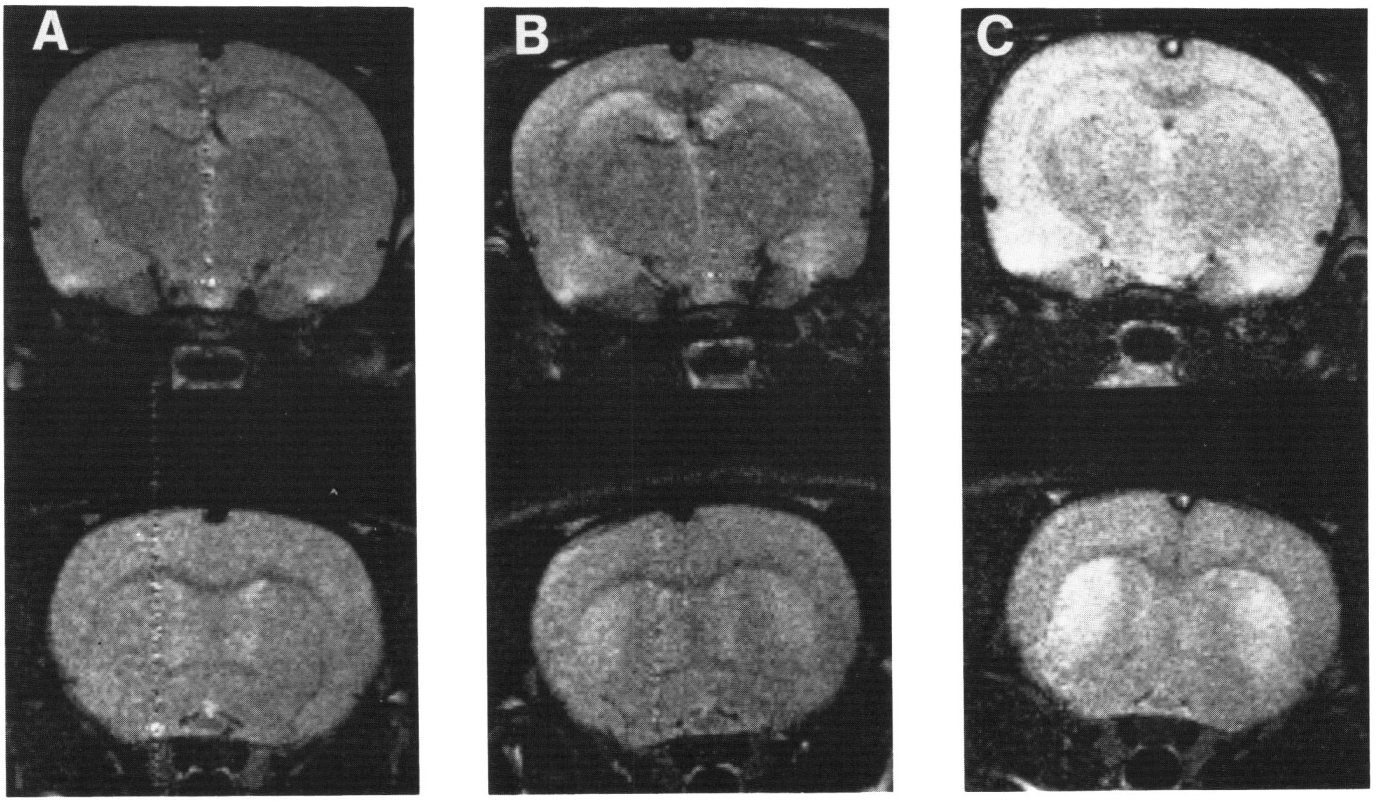

Figure 5 — Coronal MR images [TR 1365, TE66] at the level of the hippocampus (top) and striatum (bottom) of representative animals from A) the hypothermic group, obtained 2 days post-ischemia; these images are indistinguishable from images obtained pre-ischemia: B) the normothermic (38°C) group, obtained 2 days post-ischemia; note the enhanced intensity in the dorsal lateral striatum and hippocampus: C) the hyperthermic (40°C) group, obtained 1 day post-ischemia; note the uniformly enhanced intensity throughout the images.

spectroscopy data may result in delaying the activation of catabolic processes in the hypothermic animals. Furthermore, activation of these processes is accelerated in an acidic environment, so that the slower fall in tissue pH in the hypothermia animals may further delay the onset of catabolism. Together with lower rates of the catabolic reactions, this may result in the maintenance of tissue integrity and minimal edema in these animals. Accelerated catabolic activity at higher temperatures is consistent with the observations that ischemic-induced degradation of membrane phospholipids and release of eicosanoids is temperature dependent.[6,17] Furthermore, the degree of disruption of the blood brain barrier during ischemia increases with temperature.[30]

Post-ischemia, the more delayed recovery of cerebral pH in the higher temperature animals may prolong the activity of catabolic enzymes, leading to more extensive cellular injury.

The increased post-ischemia intensity in the T2 weighted MR images obtained in this study reflects increased brain water content. Histologically this edema is manifested by peri-neuronal spaces around pyknotic neurons, peri-vascular spaces, and a diffuse spongy appearance of the neuropil extending throughout the cortex and underlying white matter. The changes in both MR images and histology are most apparent and widespread in the hyperthermic group of animals, localized to the hippocampus and dorsal lateral striatum in the normothermic group, and very subtle or absent in the hypothermic animals. The development and distribution of the MR image changes parallels the histologically observed temporal and regional profile of ischemic neuronal injury and the accompanying edema.[18,19]

The results of these experiments emphasize the importance of careful control and monitoring of brain temperature in models of cerebral ischemia. Rectal temperature monitoring does not permit sufficient control of brain temperature, as the marked decrease in cerebral blood flow that accompanies most models of cerebral ischemia is accompanied by a progressive decrease in brain, but not body, temperature throughout the ischemic interval.[5,10] Monitoring and controlling the tympanic membrane/middle ear temperature, on the other hand, gives a reliable non-invasive means of accurately maintaining brain temperature. As the effects of an ischemic insult are strongly temperature dependent, it is possible that some of the inconsistencies in the results of cerebral ischemia experiments using the same animal model in different laboratories may originate from imprecise control of brain temperature.[31-32] Furthermore, various pharmaceuticals, particularly anesthetics, may compound changes in brain temperature, possibly leading to erroneous conclusions regarding the protective effects and mechanisms of action of the agents.[31-32]

This study raises several matters of clinical importance. Neurosurgical procedures are often accompanied by ischemia secondary to retractor pressure, temporary or permanent vascular occlusion, or edema. The protective effects of hypothermia described here suggest that the mild hypothermia that often accompanies general anesthetics can have a beneficial effect in ameliorating tissue injury, thereby minimizing post-operative complications. This may be enhanced by maintaining the irrigating solutions utilized during various procedures below normal

https://doi.org/10.1017/S0317167100041937 Published online by Cambridge University Press

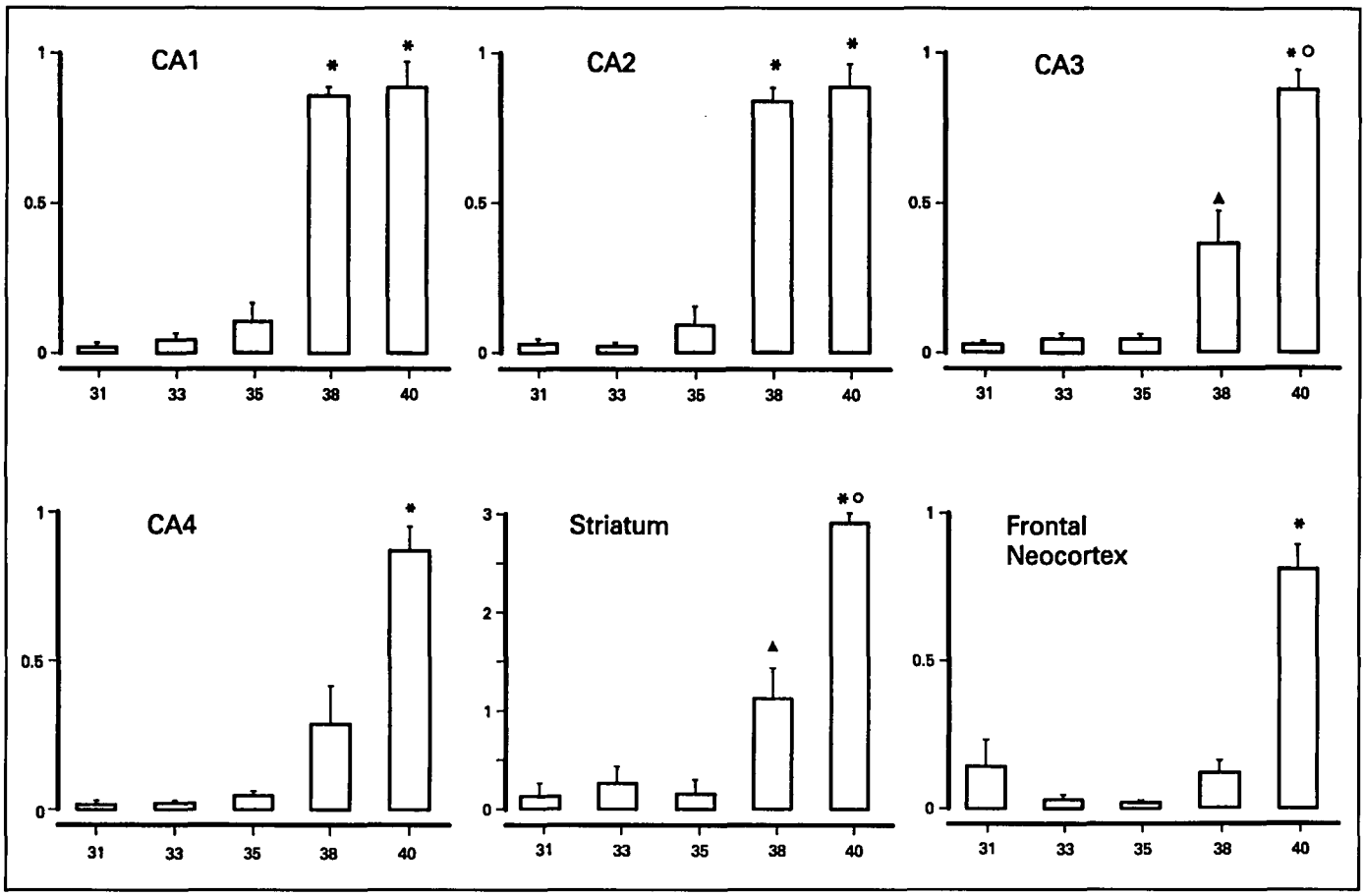

*Figure 6 — Quantitative histopathology for various brain regions following ischemia at the indicated temperature. For the hippocampal sectors and frontal neocortex, ischemic neuronal injury is expressed as the ratio (ischemic neurons/total neurons) in the standard sector. Striatal damage is graded as described in the text. For CA1 and CA2 sectors: * = $p < 0.00001$ compared to hypothermic (31, 33, 35°) groups. For CA3 sector: * = $p < 0.0001$ compared to hypothermic groups: o = $p < 0.00009$ compared to the 38°C group; and ▲ = $p < 0.02$ compared to hypothermic groups. For CA4 sector: * = $p < 0.00001$ compared to hypothermic groups and $p < 0.002$ compared to the 38°C group. For striatum: * = $p < 0.00001$ compared to hypothermic groups; o = $p < 0.00009$ compared to the 38°C group; ▲ = $p < 0.01$ compared to hypothermic groups. For frontal cortex: * = $p < 0.00001$ compared to all other groups.*

body temperature, thus possibly decreasing local brain temperature through their use. A similar argument could be made for the beneficial effects of pre-operative control of patient temperature, particularly in patients such as those with subarachnoid or intracranial hemorrhage who are at risk for ischemia. Similarly, neurosurgical procedures are often followed by mild hyperthermia. The possibility that this can aggravate ischemic tissue injury suggests that it may be prudent to control post-operative hyperthermia.

Acknowledgements

N. Haas, M. Donnelly, and R. Tyson provided valued assistance with the experiments and C. Kowalyk prepared the manuscript. Financial support was received from the Canadian Heart and Stroke Foundations and by the Winnipeg Health Sciences Centre Research Foundation.

References

1. Botterell EH, Lougheed WM, Scott JW, et al. Hypothermia and interruption of carotid or carotid and vertebral circulation in the surgical management of intracranial aneurysms. J Neurosurg 1956; 13: 1-42.
2. Botterell EH, Lougheed WM, Morley TP, et al. Hypothermia in the surgical treatment of ruptured intracranial aneurysms. J Neurosurg 1958; 15: 4-18.
3. Adams JE. Value of hypothermia and arterial occlusion in the treatment of intracranial aneurysms. Surg Gynec and Obst 1959; 108: 631-635.
4. Connolly JE, Boyd RJ, Calvin JW. The protective effect of hypothermia in cerebral ischemia: experimental and clinical application by selective brain cooling in the human. Surgery 1962; 52: 15-24.
5. Busto R, Dietrich WD, Globus MY, et al. The importance of brain temperature in cerebral ischemic injury. Stroke 1989; 20: 1113-1114.
6. Busto R, Globus MY, Dietrich WD, et al. Effect of mild hypothermia on ischemia-induced release of neurotransmitters and free fatty acids in rat brain. Stroke 1989; 20: 904-910.
7. Clifton GL, Jiang JY, Lyeth BG, et al. Marked protection by moderate hypothermia after experimental traumatic brain injury. J Cereb Blood Flow Metab 1991; 11: 114-121.
8. Dietrich WD, Busto R, Valdes I, et al. Effects of normothermic versus mild hyperthermic forebrain ischemia in rats. Stroke 1990; 21: 1318-1325.
9. Kuroiwa T, Bonnekoh P, Hossmann K-A. Prevention of post ischemia hyperthermia prevents ischemic injury of CA1 neurons in gerbils. J Cereb Blood Flow Metab 1990; 10: 550-556.

https://doi.org/10.1017/S0317167100041937 Published online by Cambridge University Press

10. Minamisawa H, Smith ML, Siesjo BK. The effect of mild hyperthermia and hypothermia on brain damage following 5, 10, and 15 minutes of forebrain ischemia. Ann Neurol 1990; 28: 26-33.
11. Welsh FA, Sims RE, Harris VA. Mild hypothermia prevents ischemic injury in gerbil hippocampus. J Cereb Blood Flow Metab 1990; 10: 557-563.
12. Cornish-Bowden A. Fundamentals of enzyme kinetics. Toronto, Butterworths and Co (Canada) Ltd 1979; 130-145.
13. Rosomoff HL, Holaday DA. Cerebral blood flow and cerebral oxygen consumption during hypothermia. Am J Physiol 1954; 179: 85-88.
14. Lougheed WM, Khan DS. Circumvention of anoxia during arrest of cerebral circulation for intracranial surgery. J Neurosurg 1955; 12: 226-239.
15. Siesjo BK. Cerebral circulation and metabolism. J Neurosurg 1984; 60: 883-908.
16. Bryan RN, Willcott MR, Schneiders NJ, et al. NMR evaluation of stroke in the rat. Am J Neuroradiol 1983; 4: 242.
17. Dempsey RJ, Combs DJ, Maley ME, et al. Moderate hypothermia reduces post ischemic edema development and leukotriene production. Neurosurgery 1987; 21: 177-181.
18. Sutherland GR, Lesiuk H, Peeling J, et al. Experimental cerebral ischemia studied using nuclear magnetic resonance imaging and spectroscopy. J Can Assoc Radiol 1990; 41: 24-31.
19. Sutherland GR, Peeling J, Lesiuk H, et al. The effects of caffeine on ischemic neuronal injury as determined by magnetic resonance imaging and histopathology. Neuroscience 1991; 42: 171-182.
20. Inoue Y, Takemoto K, Miyamoto T, et al. Sequential computed tomography scans in acute cerebral infarction. Radiology 1980; 135: 655.
21. Levy RM, Mano I, Brito A, et al. NMR imaging of acute experimental cerebral ischemia: time course and pharmacologic manipulations. Am J Neuroradiol 1983; 4: 238.
22. Peeling J, Wong D, Sutherland GR. Nuclear magnetic resonance study of regional metabolism after forebrain ischemia in rats. Stroke 1989; 20: 633-640.
23. Sutherland GR, Lesiuk H, Bose R, et al. The effect of mannitol nimodipine and indomethacin singly or in combination on cerebral ischemia in rats. Stroke 1988 19: 571-578.
24. Petroff OAC, Prichard JW, Behar KL, et al. Cerebral intracellular pH by ^{31}P nuclear magnetic resonance spectroscopy. Neurology 1985; 35: 781-788.
25. Smith ML, Auer RN, and Seisjo BK. The density and distribution of ischemic brain injury in the rat following 2-10 minutes of forebrain ischemia. Acta Neuropathol (Berl.) 1984; 64: 319-332.
26. Siesjo BK. Cell damage in the brain: a speculative synthesis. J Cereb Blood Flow Metab 1981; 1: 155-185.
27. Harris RJ, Symon L. Extracellular pH, potassium and calcium activities in progressive ischemia of rat cortex. J Cereb Blood Flow Metab 1981; 1: 203-209.
28. Yanagihara R, McCall JT. Ionic shift in cerebral ischemia. Life Sci 1982; 30: 1921-1925.
29. Cheung JY, Bonventre JV, Malis CD, et al. Calcium and ischemic injury. N Engl J Med 1986; 314: 1670-1676.
30. de-Boer J, Klein HC, Postema F, et al. Rat striatal cation shifts reflecting hypoxic-ischemic damage can be predicted by on-line impedance measurements. Stroke 1989; 20: 1377-1382.
31. Buchan A, Pulsinelli WA. Hypothermia but not the N-methyl-D-aspartate antagonist MK-801, attenuates neuronal damage in gerbils subjected to transient global ischemia. J Neurosci 1990; 10: 311-316.
32. Corbett D, Evans S, Thomas C, et al. MK-801 reduces cerebral ischemic injury by inducing hypothermia. Brain Res 1990; 514: 300-304.