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## **A Reproducible Model of Circulatory Arrest and Remote Resuscitation in Rats for NMR Investigation • Editorial Comment**

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# A Reproducible Model of Circulatory Arrest and Remote Resuscitation in Rats for NMR Investigation

Serguei Liachenko, PhD; Pei Tang, PhD; Ronald L. Hamilton, MD; Yan Xu, PhD

**Background and Purpose**—Because noninvasive physiological monitoring of cerebral blood flow, metabolic integrity, and brain ion and water homeostasis can now be accomplished with new, state-of-the-art MR spectroscopy and imaging techniques, it is appropriate to develop controllable and reproducible animal models that permit prolonged circulatory arrest and resuscitation in the magnet and also allow for studies of long-term survival and outcome. We have developed such a model in rats that involves minimal surgical preparations and can achieve resuscitation remotely within precisely controlled time.

**Methods**—Cardiac arrest was induced by asphyxiation, the duration of which ranged from 8 to 24 minutes. Resuscitation was achieved remotely by a slow, intra-aortic infusion of oxygenated blood (withdrawn either from the same rat before asphyxia or from a healthy donor rat) along with a resuscitation cocktail containing heparin (50 U/100 g), sodium bicarbonate (0.1 mEq/100 g), and epinephrine (4  $\mu$ g/100 g). The body temperature was measured by a tympanic thermocouple probe and was controlled either by a heating pad (constant tympanic temperature=37°C) or by warm ambient air (constant air temperature=37°C). Interleaved  $^{31}\text{P}/^1\text{H}$  nuclear magnetic resonance (NMR) spectroscopy was used in a selected group of rats to measure the cerebral metabolism before and during approximately 20 minutes of circulatory arrest and after resuscitation.

**Results**—The overall success rate of resuscitation, irrespective of the duration of cardiac arrest, was 82% (51 of 62). With a programmed infusion pump, the success rate was even higher (95%). The survival time for rats subjected to 15 and 19 minutes of asphyxia with core temperature tightly controlled was significantly lower than that with ambient temperature control ( $P<0.001$  and  $P<0.04$ , respectively). High-quality NMR spectra can be obtained continuously without interference from the resuscitation effort. Final histological examinations taken 5 days after resuscitation showed typical neuronal damages, similar to those found in other global ischemia models.

**Conclusions**—Because the no-flow time and resuscitation time can be precisely controlled, this outcome model is ideally suited for studies of ischemic and reperfusion injuries in the brain and possibly in other critical organs, permitting continuous assessment of long-term recovery and follow-up in the same animals. (*Stroke*. 1998;29:1229-1239.)

**Key Words:** heart arrest ■ ischemia ■ nuclear magnetic resonance ■ reperfusion ■ resuscitation ■ rats

Heart disease, the primary cause of death in the United States,<sup>1</sup> leads to cardiac arrest in many patients. The high mortality rate of cardiac arrest is due in part to our current inability to prevent and reverse tissue damage in several vital organs, particularly the brain and the heart, after a short period of ischemia. Of the 70 000 patients per year in the United States who are successfully rescued by CPR to ROSC, 60% subsequently die in the hospital; only 3% to 10% have a chance to resume their former life activities.<sup>2</sup>

To date, investigations of mechanisms of tissue damage after global ischemia have largely focused on models with reduced complexity, such as cell cultures,<sup>3</sup> perfused organs,<sup>4,5</sup> and occlusion of major vessels.<sup>6-9</sup> Although such models can provide valuable tools to define the cascade of cellular events and to identify the important components of injury process after ischemia, they often do not permit assessment of postresuscitation syndrome<sup>10-13</sup> and long-term outcome. Be-

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cause it has become clear that in the brain, and possibly in other organs, the progression and maturation of injuries after circulatory arrest occur well after the point of ROSC and are often reperfusion dependent,<sup>14</sup> development of intact animal models that precisely mimic the clinical state of cardiac arrest and resuscitation is of crucial importance for evaluation of effective postresuscitation therapy and outcome.

We report here a new cardiac arrest and resuscitation model in rats. The model involves minimal surgical preparation. Cardiac arrest and resuscitation can be controlled remotely and with high efficacy. Although the model can potentially be used in various experimental settings, it is particularly useful for state-of-the-art NMR measurements because once the animals are positioned in the magnet, there is no interference to the magnetic field homogeneity from the

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## Selected Abbreviations and Acronyms

CPR	= cardiopulmonary resuscitation
HR	= heart rate
MABP	= mean arterial blood pressure
MDPA	= methylene diphosphonic acid
NAA	= <i>N</i> -acetyl-aspartate
NDS	= neurological deficit scores
NMR	= nuclear magnetic resonance
pH <sub>i</sub>	= intracellular pH
ROSC	= restoration of spontaneous circulation

resuscitation efforts. High-quality imaging and spectroscopic measurements are thus possible throughout the crucial periods of cardiac arrest, resuscitation, and subsequent recovery.

## Materials and Methods

The experimental protocol was approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh. Male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, Ind), weighing  $164 \pm 33$  g, were used. Younger rats were chosen for their relevance to asphyxial cardiac arrest in infants and children. Sixty-six rats were randomized into 10 groups (Table 1). In group 1 (sham operation), rats were surgically prepared, as described below, but were not subjected to asphyxia and cardiac arrest. In groups 2 through 7, rats were respectively subjected to 8, 8, 10, 12, 15, and 19 minutes of asphyxia. In these groups, the rat body temperature, measured by a tympanic temperature probe, was precisely controlled to  $37.0 \pm 0.5^\circ\text{C}$  throughout the experiment, with the use of a feedback-controlled heating pad (temperature control method I). Groups 8, 9, and 10 underwent 15, 19, and 24 minutes of asphyxia, respectively, with body temperature controlled by a flow of  $37.0^\circ\text{C}$  ambient air (temperature control method II). This second method of temperature control was tested because of its clinical relevance, despite the fact that it was insufficient to maintain a constant tympanic temperature during prolonged circulatory arrest.

## Animal Preparation

In a typical experiment, rats were anesthetized with 4% isoflurane in an O<sub>2</sub> (30%) and N<sub>2</sub>O (70%) mixture and intubated orotracheally.

After intubation, anesthesia was reduced to 1.25% isoflurane in an O<sub>2</sub> (30%) and air (70%) mixture, and the rats were paralyzed with pancuronium bromide (0.02 mg/100 g) and mechanically ventilated with a tidal volume of 1 mL/100 g, 40 breaths per minute, and a positive end-expiratory pressure of 5.5 cm H<sub>2</sub>O. Baseline arterial pH was maintained at  $\approx 7.4$ , PaCO<sub>2</sub> at  $\approx 30$  mm Hg, and PaO<sub>2</sub>  $> 170$  mm Hg. Both femoral arteries and the left femoral vein were cannulated. One of the arterial catheters was attached to a pressure transducer (Baxter Edwards, model 53-DTS-260), which was interfaced through a Grass 7D Polygraph recorder (Grass Instrument Co) to a PowerMac 8100 computer (Apple Computer, Inc) running the LabVIEW program (National Instruments) for continuous display and recording of arterial blood pressure, arterial pulse pressure, and HR. The other arterial catheter was slowly advanced through the abdominal aorta to the thoracic aorta for later resuscitation use (see below) and for arterial blood sampling. The arterial blood gases and pH were determined every 60 minutes with a Corning 178 pH/blood gas analyzer (Corning Medical and Scientific). A conventional six-lead ECG was recorded with the Grass 7D Polygraph recorder. The tympanic membrane temperature was measured by a flexible thin thermocouple inserted deeply into the left auditory canal, which was then closed with cotton.

## Cardiac Arrest and Resuscitation

Five minutes before induction of asphyxial cardiac arrest, rats received a dose of the short-acting muscle relaxant vecuronium bromide (0.01 mg/100 g IV, plus 50 U/100 g heparin) to prevent spontaneous breathing during asphyxia. A minute before asphyxia, a small amount of oxygenated arterial blood (1 mL/100 g body wt) was collected into a syringe (groups 1 and 3 to 10). Group 2 differed from others in that no blood was withdrawn from the rat under study but rather from a healthy donor, of the same litter, 1 minute before the planned resuscitation. The syringe collecting the blood contained a mixture of heparin (50 U/100 g), sodium bicarbonate (0.1 mEq/100 g), and epinephrine (4  $\mu\text{g}$ /100 g) in a total volume of 0.25 mL/100 g body wt. Asphyxiation was caused by disconnecting the ventilation tubes from the ventilator and clamping them. This led to electromechanical dissociation, as indicated by a decrease in MABP to  $< 10$  mm Hg and an arterial pulse pressure to  $< 5$  mm Hg, and subsequent pulseless electric activity. Resuscitation began with 100% O<sub>2</sub> ventilation and a slow infusion of the oxygenated arterial blood into the thoracic aorta. In earlier experiments the infusion was

TABLE 1. Summary of Cardiac Arrest and Resuscitation Variables

Group	No. of Rats (ROSC/Total)	Time Intervals Between Events, min*				Mean Survival Time, † h	NDS of Survived Rats (No. of Rats)
		II–IV	II–III	IV–V	III–V		
Temperature control method I							
1	4/4	...	...	...	...	>120	496 ± 5 (4)
2	5/5	8	1.3 ± 0.3	1.9 ± 0.3	8.6 ± 0.5	87 ± 18	498 ± 2 (3)
3	6/7	8	2.7 ± 0.7	1.9 ± 0.9	7.1 ± 1.3	71 ± 20	443 ± 74 (3)
4	4/4	10	2.2 ± 1.1	1.9 ± 0.7	9.8 ± 1.3	68 ± 27	408 ± 131 (2)
5	4/6	12	3.3 ± 1.2	2.2 ± 0.7	11.0 ± 1.7	34 ± 9§	...
6	6/9	15	3.6 ± 1.0	3.1 ± 0.5	14.1 ± 1.4	22 ± 5§	...
7	2/4	19	2.6 ± 0.6	2.2 ± 0.2	19.1 ± 0.3	2 ± 0§¶	...
Temperature control method II							
8	5/7	15	3.5 ± 0.6	2.0 ± 0.5	14.3 ± 0.9	>120	467 ± 45 (5)
9	12/13	19	2.5 ± 0.9	2.8 ± 0.7	20.0 ± 0.7	86 ± 14¶	478 ± 15 (8)
10	7/7	24	2.5 ± 0.8	3.9 ± 1.5	25.4 ± 1.3	28 ± 15§	490 ± 1 (1)

\*Events II through V are defined in Figure 2.

†Calculated by Kaplan-Meier Survival Analysis.

‡Only one rat survived for  $> 5$  days in this group, so that SD is not displayed.

§Significantly different from group 1 ( $P < 0.01$ , Duncan multiple comparison).

||Significantly different between the two groups ( $P < 0.001$ , log-rank test).

¶Significantly different between the two groups ( $P < 0.04$ , log-rank test).

done manually, but for the 19 rats in the most recent experiments it was accomplished automatically at an infusion rate of 0.45 mL/100 g per minute with the use of a programmable syringe pump (KDS-210, KD Scientific Inc). For group 2, the same volume of venous blood was collected during infusion to maintain the hemodynamic balance.

With this intra-aortic infusion method, resuscitation to ROSC could be achieved without electric defibrillation or chest compression. ROSC was determined as the point at which MABP reached a value  $>60$  mm Hg with a supraventricular cardiac rhythm. In group 2, donor blood infusion was discontinued at the point of ROSC. In all groups, if ROSC was impossible after all withdrawn blood had been returned, no further resuscitation was attempted. The resuscitated rats were continually ventilated for  $\geq 2$  hours with a mixture of O<sub>2</sub> (30%) and air (70%). Anesthesia was reinstated with 1% isoflurane if signs of awakening were observed or if HR increased to  $\geq 300$  beats per minute.

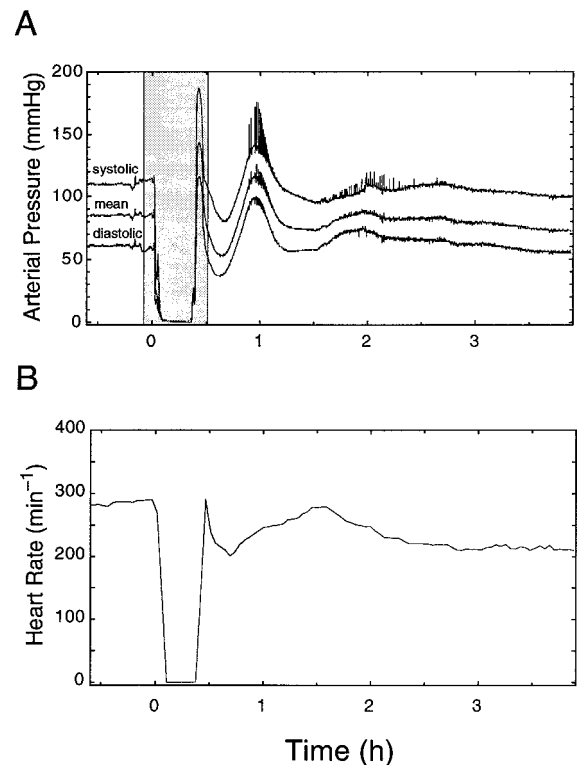
At least 2 hours after resuscitation and under isoflurane general anesthesia, the arterial and venous catheters were surgically removed and the wound was closed. Rats were then mechanically ventilated with room air and allowed to extubate themselves. After extubation, they were returned to their cages and monitored closely for long-term outcome evaluation. Whenever needed, rats were given fluids subcutaneously or fed with ground chew with water. Rats that did not extubate themselves, or later became morbid or could not attend to their physiological needs, were killed and prepared for histological examination.

## Outcome Evaluation

Animals were closely observed for 3 to 5 days after resuscitation. In animals that survived for  $>3$  days, the neurological deficit scores (NDS) were evaluated by the same investigator using the criteria proposed by Neumar and coworkers.<sup>15</sup> After final NDS evaluation, animals were reanesthetized (1.5% isoflurane through a nose cone mask). A thoracotomy was performed, and a 14-gauge catheter was advanced into the aortic arch through the apex of the left ventricle. The descending aorta was then clamped. Neutrally buffered 3% paraformaldehyde was infused through the catheter under a pressure of 100 cm H<sub>2</sub>O. The right atrium was incised, and perfusion was continued until the fluid draining from the right atrium was clear (approximately 60 mL). The rat was then decapitated, and the head was stored in 3% paraformaldehyde for 24 hours. The brain was then removed from the skull and stored in the fixative. Paraffin-embedded coronal sections, 6  $\mu$ m thick, were made through the level of hippocampus and stained with hematoxylin-eosin.

## Interleaved <sup>31</sup>P/<sup>1</sup>H NMR Spectroscopy

To demonstrate the compatibility of the model with NMR investigation, 6 rats from group 9 were subjected to continuous interleaved <sup>31</sup>P/<sup>1</sup>H NMR spectroscopic measurements before, during, and after circulatory arrest and resuscitation. Experiments were performed with an Otsuka CMXW-400SLI spectrometer, equipped with a 9.4-T, 111-mm bore magnet. Because of the vertical orientation of the magnet, rats were snugly positioned, head up, in a specially designed cradle. A double-resonance surface-coil probe was used, consisting of an 11 $\times$ 13-mm elliptical surface coil tuned to 162.367 and 401.102 MHz for <sup>31</sup>P and <sup>1</sup>H resonance frequencies, respectively. The pulse sequences for interleaved acquisition consisted of a conventional one-pulse sequence for <sup>31</sup>P and a spin-echo sequence with gaussian water suppression for <sup>1</sup>H. The pulse widths for <sup>31</sup>P and <sup>1</sup>H were 32 and 40 microseconds, respectively, which produced nominal 90° flip at a position 7 mm perpendicular from the center of the surface coil. Spin-echo time for <sup>1</sup>H was set to 136 milliseconds to maximize water suppression and lactate detection.<sup>16</sup> Data were acquired in 4096 complex points with a spectral width of 10 kHz for a pair of interleaved <sup>31</sup>P and <sup>1</sup>H spectra. Interleaving was achieved by acquiring data from one nucleus during the mandatory relaxation period of the other. Additional relaxation time was added to ensure a repetition delay of 1.8 seconds for <sup>31</sup>P and 1.6 seconds for <sup>1</sup>H. A total of 152 scans were summed and zero-filled once before Fourier



**Figure 1.** Representative cardiovascular response to 19 minutes of asphyxiation (from group 9). Traces of systolic, mean, and diastolic arterial blood pressure (A) and HR (B) before, during, and after cardiac arrest and resuscitation are displayed as a function of time. Time 0 is arbitrarily assigned to the moment immediately before the arterial blood withdrawal.

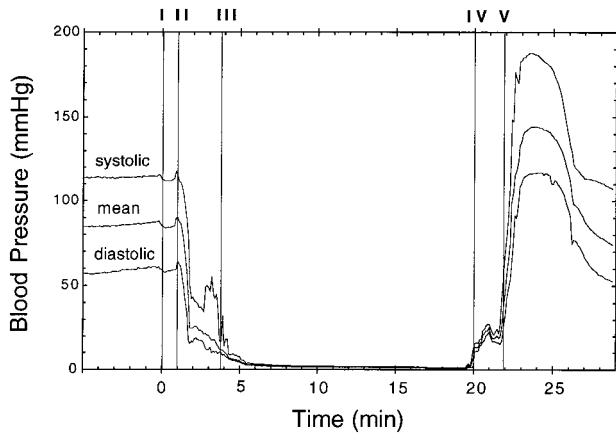
transform. The temporal resolution for a pair of interleaved spectra was  $\approx 5$  minutes.

## Data Analysis

Statistical analysis was performed with the use of the SPSS program (SPSS Inc). Simple factorial two-way ANOVA was used to determine the effects of asphyxia time, temperature control method, and rat orientation on long-term survival, NDS, and HR. Repeated-measures ANOVA was used to compare the physiological variables among groups at three time points: before cardiac arrest and 1 and 2 hours after resuscitation. If a significant change was indicated for a given variable, then Student-Newman-Keuls multiple comparisons were made to determine the differences between time points within groups or between groups at a given time point. Differences in NDS among groups were compared with the nonparametric Kruskal-Wallis test. Survival of rats was analyzed with Kaplan-Meier Survival Analysis with the log-rank test for between-group comparisons. The mean survival times were also compared among all groups with the Duncan multiple comparisons test. All data are expressed as mean  $\pm$  SD.

## Results

Figure 1 shows the representative traces of arterial blood pressure and HR before, during, and after cardiac arrest and resuscitation from a rat in group 9 (19 minutes of asphyxia). The arterial blood pressures in the shaded time interval in Figure 1 are expanded in Figure 2 to indicate several key events, labeled I through V. The withdrawal of arterial blood (interval I-II) led to a slight ( $<4\%$ ) decrease in MABP. The time from the onset of asphyxia to electromechanical dissociation (interval II-III) was  $2.88 \pm 0.95$  minutes, averaged



**Figure 2.** Detailed changes in arterial blood pressure during the shaded interval in Figure 1A. Important events are as follows: I, beginning of blood withdrawal; II, onset of asphyxia; III, beginning of no-flow by definition; IV, beginning of resuscitation; and V, ROSC by definition. The duration of asphyxia and that of circulatory arrest are defined by the intervals II-IV and III-V, respectively.

among 57 rats in groups 3 to 10, and was  $1.33 \pm 0.31$  minutes for group 2 (no preasphyxia blood withdrawal). Event III marks the beginning of circulatory arrest by definition. The infusion of oxygenated blood and reventilation (event IV) resulted in an MABP increase, the initial rate of which was controlled mainly by the rate of infusion. A rapid elevation in MABP, leading to ROSC (event V by definition), occurred either during or immediately after completion of the infusion in 51 of 62 rats (a success rate of 82%). The success rate with pump resuscitation was even higher and seemed independent of the no-flow time. Of the 19 rats resuscitated by the pump, only one did not show ROSC after all shed blood was returned. Table 1 summarizes the number of resuscitated rats of the total rats in each group, the intervals between the major events, the mean survival time, and the NDS measured 3 days after resuscitation. Note that the duration of asphyxia and that

of circulatory arrest (no-flow time) are indicated in Table 1 by II-IV and III-V, respectively.

The MABP and HR immediately before arterial blood withdrawal or 1 minute before asphyxia for group 2 (arbitrarily assigned as time 0) and 1, 2, and 3 hours after ROSC are listed in Table 2. Corresponding data from group 1, with time 0 assigned to the point half an hour after surgical preparation and before blood withdrawal, are also included. MABP showed a similar decrease after cardiac arrest in all animals. The HR was significantly lower in groups 9 and 10 at 2 hours after ROSC compared with the sham-operated rats (group 1).

Other important physiological variables are listed in Table 3 for all groups. After 24-minute asphyxiation (groups 7 and 10), any attempts to take an arterial blood sample after ROSC caused a significant decrease in MABP. Therefore, no blood sample was taken for these two groups after ROSC. In other groups, arterial blood pH showed a tendency to recover soon after ROSC. Only group 9 showed a significantly lower value at 1 hour after ROSC compared with the sham operation. No significant changes in  $Paco_2$  and  $Pao_2$  were found.

Representative ECG tracings (lead II) for groups 3 through 10 are displayed in Figure 3. Electric silence was not observed after the mechanical asystole. In groups with prolonged asphyxia (19 and 24 minutes), however, ventricular complexes disappeared 3 to 7 minutes before resuscitation. In almost all rats, the electric activity of the heart reappeared immediately after the onset of resuscitation procedure and intensified before ROSC. In groups 3 and 8, ECG returned to normal 2 hours after ROSC and remained normal 3 to 5 days after resuscitation. All other groups showed different extents of ECG abnormalities, indicating certain degree of ischemic and hypoxic damage to the myocardium.<sup>17</sup>

All sham-operated rats (group 1) and all resuscitated rats in group 8 were alive 5 days after the episode. When the tympanic membrane temperature was precisely controlled to 37°C throughout the experiments, the percentages of  $\geq 3$ -day survival of the successfully resuscitated rats were 60% for

**TABLE 2. Cardiovascular Variables Before Cardiac Arrest and After Resuscitation**

Group	MABP, mm Hg*				HR, bpm			
	Before CA	1 h After ROSC	2 h After ROSC	3 h After ROSC	Before CA	1 h After ROSC	2 h After ROSC	3 h After ROSC
1	106±18	98±16	91±15	93±7	405±24	403±37	405±46	409±33
2	109±7	110±12	102±11	103±8	385±27	331±95	299±13	301±17
3	117±11	98±12	98±12	86±13	435±19	398±44	378±52	322±26
4	107±11	76±8†	84±19	79±13	412±53	450±56	379±23	365±7
5	114±12	89±5†	86±27†	85±22	427±47	441±39	390±23	369±20
6	126±16	111±20	83±20†	79±25	397±24	397±37	348±36	379±17
7	120±19	102±3	87±11†	...	443±21	410±72	315±100	...
8	117±13	92±20	92±16	95±14	373±51	378±62	349±60	345±57
9	100±6	80±7†	76±11†	71±12	358±29	398±126	280±23††	270±21
10	110±11	73±10†	72±24†	67±13	332±37	300±86	264±73††	249±112

\*MABP is not intended for comparison between different groups because of seasonal changes in normal MABP, as well as difference in bench-top and in-magnet experiments.

†Significantly different ( $P < 0.05$ ) from the initial level (before cardiac arrest [CA]) within the same group.

‡HR significantly different ( $P < 0.05$ ) from group 1.

**TABLE 3. Arterial Blood pH and Blood Gases Before Cardiac Arrest and After Resuscitation**

Group	pH			Paco <sub>2</sub> , mm Hg			PaO <sub>2</sub> , mm Hg		
	Before CA	1 h After ROSC	2 h After ROSC	Before CA	1 h After ROSC	2 h After ROSC	Before CA	1 h After ROSC	2 h After ROSC
1	7.41±.04	7.43±.02	7.37±.11	23.7±4.4	30.2±5.1	32.7±10.8	192±23	183±44	195±16
2	7.39±.02	7.33±.02	7.32±.02	32.9±7.2	41.7±3.5	32.0±4.0	190±15	199±19	209±20
3	7.45±.03	7.37±.04	7.42±.03	26.9±4.3	31.3±6.8	30.5±5.1	180±23	148±48	181±18
4	7.40±.06	7.41±.01	7.31±.19	29.8±7.8	29.6±8.1	30.6±24.0	177±36	169±25	146±67
5	7.43±.04	7.33±.01	7.36±.02	29.0±5.0	31.0±11.6	30.4±7.0	182±33	196±21	192±18
6	7.39±.08	7.33±.04	7.37±.02	32.5±10.1	39.7±3.5	29.7±6.4	212±34	179±5	206±26
7	7.39±.03	...*	...*	28.3±4.6	...*	...*	188±45	...*	...*
8	7.47±.03	7.33±.10	7.36±.06	18.0±4.7	23.1±9.1	26.1±8.1	218±15	208±40	196±13
9	7.45±.03	7.06±.12†	7.28±.03	24.4±5.9	40.2±11.1	31.6±5.4	211±7	180±58	180±31
10	7.44±.04	...*	...*	29.9±4.7	...*	...*	212±16	...*	...*

CA indicates cardiac arrest.

\*In groups 7 and 10, any attempt to take a blood sample after ROSC caused a significant decrease in MABP. Therefore, no blood samples were taken after ROSC.

†Significantly different ( $P<0.05$ ) from the sham at the same time point.

group 2 (3 of 5), 50% for group 3 (3 of 6) and group 4 (2 of 4), and 0% for groups 5, 6, and 7 (0 of 4, 6, and 2, respectively). In contrast, when the body temperature was controlled with 37°C ambient air (the tympanic temperature was gradually decreased to 34°C to 35°C during cardiac arrest), the long-term survival was significantly better. The percentage of  $\geq 3$ -day survival was 100% for group 8 (5 of 5) and 67% for group 9 (8 of 12). However, after prolonged 24-minute asphyxiation (group 10), the mean survival time was significantly lowered.

For rats that remained alive  $\geq 3$  days after the surgery (group 1) or ROSC (groups 2 to 4 and 8 to 10), there was no significant difference in NDS ( $P>0.63$ ). Rats that died  $< 3$  days after resuscitation were excluded from the NDS evaluation. The reduction in the NDS primarily reflected impairment to the hind limbs. It was not determined whether this impairment was due to neurological damage or to surgical procedures.

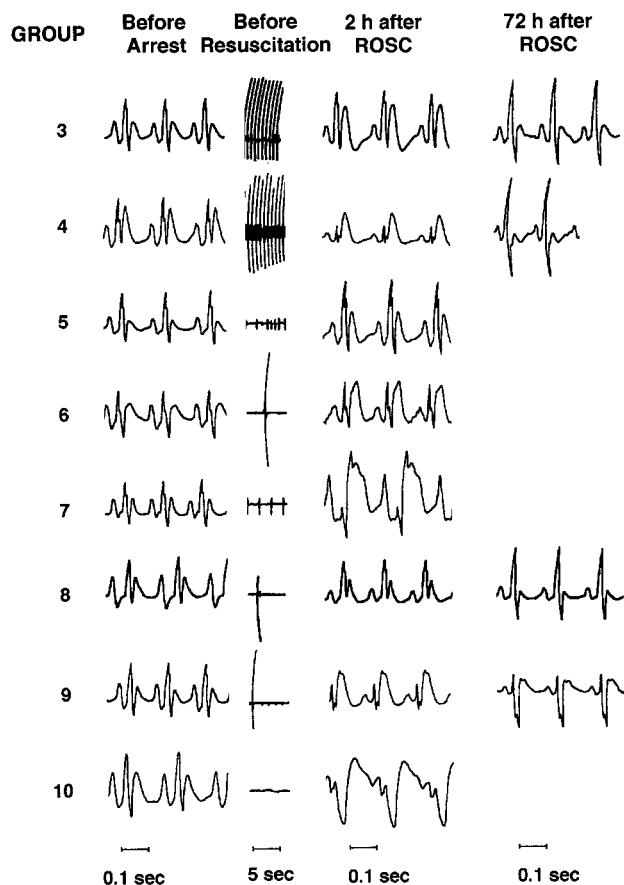
Because of the orientation of our magnet, rats had to be positioned vertically for NMR measurements. To determine the effects of orientation, we assigned 41 rats to be positioned vertically in the NMR probe and 25 horizontally on the bench top. Two-way ANOVA with respect to asphyxia time, temperature control method, and rat orientation showed that the orientation had no significant effects on long-term survival ( $P>0.79$ ), NDS ( $P>0.82$ ), and HR before ( $P>0.17$ ) and after ( $P>0.07$ ) cardiac arrest.

Figure 4A shows representative interleaved  $^{31}\text{P}/^1\text{H}$  NMR spectra before, during, and after 20 minutes of circulatory arrest. The peaks assigned are ATP, phosphocreatine, inorganic phosphate, phosphomonoester, and MDPA in the  $^{31}\text{P}$  spectra, and NAA, creatine, choline, glutamate/glutamine, and lactate in the  $^1\text{H}$  spectra.  $\text{pH}_i$  can be calculated on the basis of chemical shifts in the  $^{31}\text{P}$  spectra.<sup>18</sup> The MDPA peak was from a sealed external reference, whose position relative to the NMR coil was fixed. Detailed changes in ATP, phosphocreatine, inorganic phosphate,  $\text{pH}_i$ , NAA-choline ratio, and lactate, averaged for six rats in group 9, are depicted

in Figure 4B. As can be seen, immediately after cardiac arrest phosphocreatine was completely depleted; ATP decreased to  $< 20\%$  of control;  $\text{pH}_i$  was lowered to 6.2; the NAA-choline ratio decreased gradually during the  $\approx 20$  minutes of circulatory arrest and continued to decline after ROSC for  $\approx 30$  minutes to 70% of the control; inorganic phosphate increased by  $> 500\%$ ; and lactate increased by 340%. The residual ATP during the cardiac arrest is believed to arise from the slightest signal contamination from the extracranial muscle. Because surgery was minimized in this outcome model, the extracranial muscle was not extracted.

After resuscitation, inorganic phosphate and phosphocreatine recovered to the control level within 30 and 80 minutes after ROSC, respectively. ATP, however, did not fully recover until 2 hours after ROSC. The NAA-choline ratio recovered slowly over a course of 3 hours but only to 90% of the control level, suggesting a certain degree of neuronal damage. Recovery of  $\text{pH}_i$  was biphasic in this particular group of rats; there was a rapid return of  $\text{pH}_i$  to 7.1, which correlated in time with the clearance of lactate, and a slow normalization over a period of 2 to 3 hours.

Figure 5A depicts a typical section of hippocampus from a rat 5 days after an 8-minute cardiac arrest (from group 3). The neurons of the CA1 region showed typical hypoxic/ischemic changes, similar to those found in other global ischemia models. These changes included vacuolization and hyperosinophilia of the neuronal cytoplasm, nuclear pyknosis, and karyorrhexis. Injuries were only rarely present in the cortical neurons and were not present in other portions of the hippocampal formation. In rats killed 3 days after cardiac arrest, only rare neurons in this area showed eosinophilia, but there was considerable vacuolization in some cases (Figure 5B). None of these changes were seen in rats killed within 24 hours of cardiac arrest (Figure 5C). In animals in which method II of temperature control was used, only vacuolization of cytoplasm could be seen in the CA1 region 3 days after ROSC.



**Figure 3.** Representative ECG tracing in rats before, during, 2 hours, and 3 days after cardiac arrest. There was no immediate electric silence in ECG after mechanical asystole, indicating electric-mechanical dissociation. In most rats in the prolonged asphyxiation groups ( $\geq 19$  minutes of asphyxia), ventricular complexes disappeared before the resuscitation procedure had begun. ROSC was preceded by restoration of normal supraventricular cardiac rhythm. ECG tend to recover after mild ischemic insult (groups 3 and 8) but showed hypoxic/ischemic myocardial damage in other groups.

### Discussion

A highly reproducible cardiac arrest and resuscitation model in rats is presented. The model is developed specifically for use with noninvasive MR spectroscopic and imaging techniques, but clearly it can be used for other studies in which remotely controlled resuscitation and minimal animal movement during data acquisition are required. In addition to the ease of control of the no-flow and resuscitation times, a particular advantage of the present model for MR investigation is its minimal (essentially none) perturbation to the magnetic field homogeneity during resuscitation. Unlike conventional CPR or electric defibrillation in which unpredictable animal movements due to CPR or the noise and secondary field caused by the electric current can interfere with magnetic field homogeneity, the present resuscitation method permits uninterrupted MR measurements during the most crucial period of circulatory arrest and reperfusion. Moreover, because of its clinical relevance, the model is ideally suited for investigation of the mechanisms of post-resuscitation syndrome,<sup>10</sup> such as perfusion failure in the

brain and possibly other organs, reoxygenation injury leading to cell necrosis, extracerebral organ derangement, and blood derangement. The feasibility of long-term survival in this model also allows for assessment of possible postarrest pharmacological interventions and follow-up studies during the crucial period of recovery after prolonged circulatory arrest.

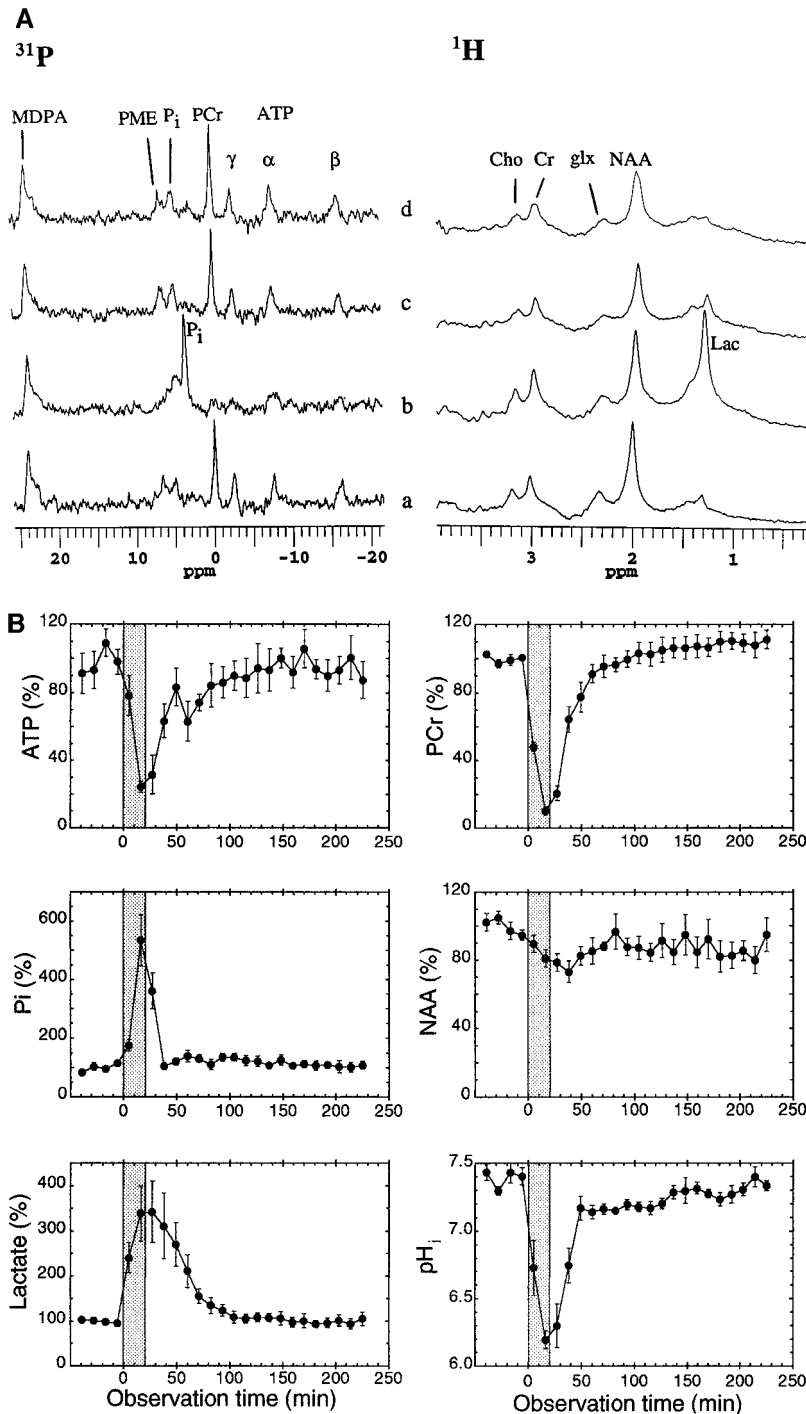
Although asphyxia differs from ventricular fibrillation as the primary cause for circulatory arrest, the postarrest resuscitation and the evaluation of reperfusion injury and delayed neuronal death are similar. Moreover, the asphyxia-induced cardiac arrest is, in its own right, of great clinical importance: coma-induced hypoventilation is the second most common indication for CPR and cerebral resuscitation, and in children the predominant cause of cardiac arrest is asphyxia.<sup>19</sup>

The resuscitation regimen used in the present model (ie, 2- to 3-minute infusion of oxygenated arterial blood directly into the thoracic aorta) resembles closely the procedures for cardiopulmonary bypass, implicating the potential clinical importance of the model. Partial cardiopulmonary bypass (or partial cardiopulmonary support<sup>20</sup>) is routinely used in clinical practice for extracorporeal life support in humans and sometimes also for experimental resuscitation.<sup>21-25</sup> Although intra-arterial administration of medication (eg, epinephrine) should be practiced with caution, the low doses used in the model can be further reduced when donor blood is used (such as in group 2), because the withdrawn donor blood and predetermined dose of medication need not be infused after ROSC is observed. As an experimental approach, our intra-aortic blood infusion proves to be a very reliable method for remotely controlled resuscitation after prolonged cardiac arrest.

Except for group 2, the amount of heparin administered was high. Although we observed no evidence of hemorrhage after resuscitation, heparin may alter hemodynamic, metabolic, and immunologic responses after resuscitation from cardiac arrest. In our recent modification of the method using donor rats, we tested the use of heparin-coated extracorporeal devices<sup>26</sup> without administering free heparin. Excellent resuscitation was obtained (data not shown), and no blood coagulation or microclots were found in the coated devices. Numerous studies<sup>27-29</sup> in which the same type of coating in cardiopulmonary bypass was used have shown greatly improved outcome. Release of heparin from the coating was virtually undetectable.<sup>26</sup>

Successful resuscitation after prolonged cardiac arrest of up to 32 minutes has been demonstrated previously with the use of extracorporeal circulation,<sup>30</sup> two-stage resuscitation,<sup>31</sup> or internal cardiac massage.<sup>32</sup> Not all of these, however, permit evaluation of animal survival and neurological outcome. In one study in dogs, after 20 minutes of ventricular fibrillation followed by resuscitation with cardiopulmonary bypass,<sup>23</sup> nine of 10 dogs were alive 72 hours after arrest, but none regained normal neurological function at 96 hours after arrest.

In earlier models of asphyxial cardiac arrest in rats, resuscitation was achieved by extrathoracic compression either manually<sup>11,15</sup> or with the use of a remotely controlled pneumatic balloon.<sup>33</sup> The present model has an advantage

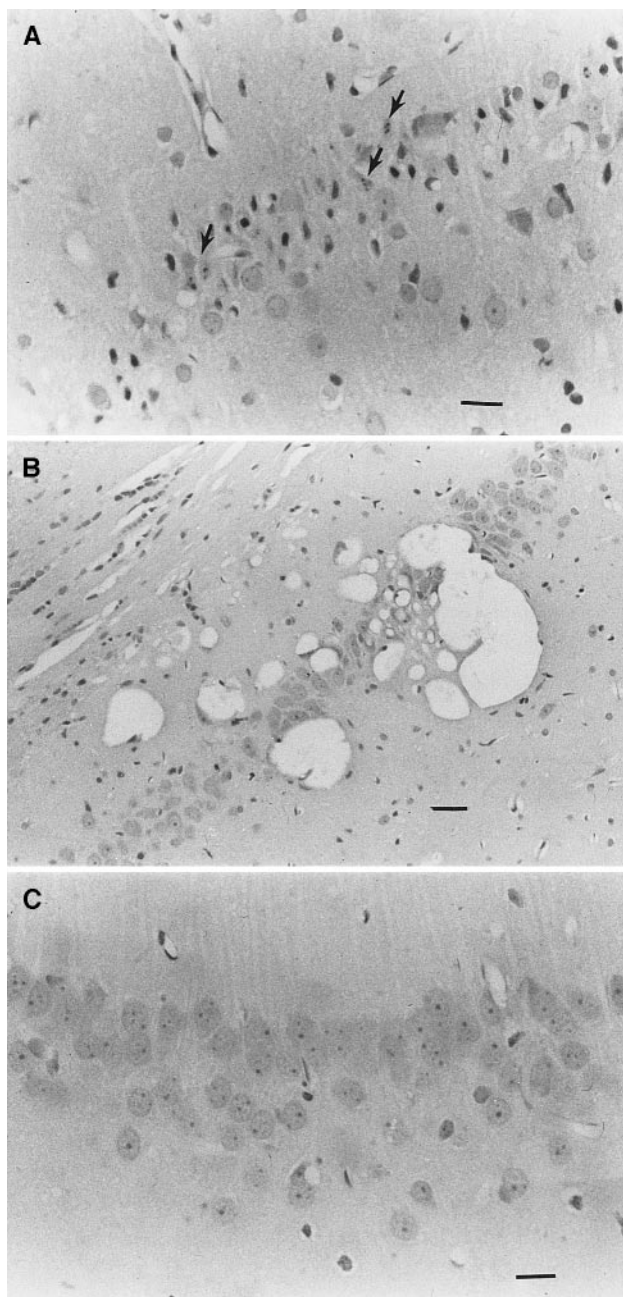


**Figure 4.** A, Representative interleaved  $^{31}\text{P}/^1\text{H}$  NMR spectra of a rat brain before (a) and during (b) 20 minutes of cardiac arrest (group 9) and 1 hour (c) and 3 hours (d) after resuscitation. B, Time course of brain metabolic changes (n=6) before, during, and after 20 minutes of asphyxial cardiac arrest.  $\text{pH}_i$  was calculated from the parts-per-million differences in resonance frequencies between inorganic phosphate ( $\text{P}_i$ ) and phosphocreatine (PCr) ( $\delta$ ), according to  $\text{pH}_i = 6.683 + \log[(\delta - 3.153)/(5.730 - \delta)]$ .<sup>16,18</sup> PME indicates phosphomonoester; Cho, choline; Cr, creatine; glx, glutamate/glutamine; and Lac, lactate.

over these earlier models in that the time from the beginning of resuscitation to ROSC can be precisely controlled by the rate at which the oxygenated arterial blood is infused, thus making the no-flow time reproducible. This will ensure a similar degree of insult to all animals subjected to the same period of asphyxia, so that results from different animals can be compared.

Intravenous injection of 0.5 mol/L KCl has been used in a transient cardiac arrest model in rats,<sup>34</sup> in which  $\approx 2\%$  venous blood (milliliters per gram body weight) was withdrawn after KCl injection, and rats were resuscitated 3.5 minutes later by

external cardiac compression and intravenous blood infusion. Although KCl injection causes isoelectric ECG almost instantly and thus offers a good control of the time to induce cardiac arrest, resuscitation after KCl-induced cardiac arrest requires CPR<sup>34,35</sup> and is impossible without electric defibrillation if no-flow time is prolonged (unpublished data, Y. Xu, J. Melick, 1994). Any large variation in resuscitation time defeats the purpose of having a tight control of the arrest time in the first place. Moreover, KCl poisoning is a rather rare medical problem; the clinical implication of KCl-induced cardiac arrest is thus not as clearly defined as that of asphyxial cardiac arrest.



**Figure 5.** A, Section of hippocampus from a rat 5 days after 8-minute cardiac arrest. The neurons of the CA1 region show hypoxic/ischemic changes, including hypereosinophilia and vacuolization of the neuronal cytoplasm, nuclear pyknosis, and karyorrhexis (arrows). These changes are only rarely present in cortical neurons and are not present in other portions of the hippocampal formation. B, In rats killed 3 days after cardiac arrest, some show vacuolization in the CA1 region but only rare eosinophilic neurons. C, None of the changes can be found in rats killed within 24 hours of cardiac arrest. Hematoxylin-eosin-stained sections of paraffin-embedded tissue. Bar=10  $\mu\text{m}$  in A and C; bar=20  $\mu\text{m}$  in B.

When our model is compared with other global ischemia models, it should be noted that the present study uses relatively young rats, chosen for their relevance to asphyxial cardiac arrest in infants and children. Although not investigated here, the age of the animal may affect the resuscitation and survival rates. However, in terms of excitotoxicity, the

rats used in the present study ( $164 \pm 33$  g; 46 to 50 days old) are comparable to adult rats. Recent studies using intracerebral microinjection of neurotoxins<sup>36,37</sup> have shown that in rats peak vulnerabilities to *N*-methyl-D-aspartate,  $\alpha$ -amino-3-hydroxy-5-methyl-4 isoxazole propionic acid (AMPA), and kainate occur at postnatal days 6, 10, and 21, respectively, and the sensitivity to excitotoxic damage flattens after the age of 21 days.

The body temperature during and after cardiac arrest and resuscitation has a profound impact on the severity of injury and long-term outcome.<sup>24,38–40</sup> The two temperature control methods used in this study (viz, precise control with a feedback heating device wrapped around the rats to maintain the tympanic membrane temperature at 37°C and the ambient air control to maintain the ambient temperature at 37°C) resulted in significantly different outcomes after prolonged cardiac arrest. For example, when group 6 is compared with group 8 (both were subjected to 15 minutes of asphyxia, resulting in 14 minutes of circulatory arrest) and group 7 is compared with group 9 (19 and 20 minutes of circulatory arrest, respectively), the mean survival time was significantly shorter ( $P < 0.001$  for 15 minutes and  $P < 0.04$  for 19 minutes) for rats in which method I was used (Table 1). Although the ambient temperature was controlled at 37°C, method II was insufficient to maintain a constant rat body temperature. In the case of group 9 (19 minutes of asphyxia), the rat body temperature was gradually decreased to 34°C toward the end of circulatory arrest. Correspondingly, the histological changes in the vulnerable CA1 region of the hippocampus were less expressed in animals with the temperature control of method II (Figure 5B).

The issue of temperature control in studies of cardiac arrest and resuscitation should be viewed from two different perspectives. First, precise temperature control is necessary for mechanistic studies, especially when the effects of therapeutic agents are under investigation. However, temperature control with ambient air, allowing the body temperature to decrease naturally during circulatory arrest, is more closely relevant to a clinical setting. Our two methods of controlling temperature can be selected to ideally meet both needs.

No special treatment was administered after ROSC to correct the arterial pH in the present model. The <sup>31</sup>P NMR data showed that brain pH<sub>i</sub> was lowered from the control level of 7.4 to 6.2 during cardiac arrest in group 9, recovered to 7.1 immediately after ROSC, stayed at this level for  $\approx 2$  hours, and then gradually returned to the control level. It has been suggested<sup>41</sup> that reduction of the extracellular HCO<sub>3</sub><sup>-</sup> concentration greatly inhibits the rate of pH<sub>i</sub> recovery and that correction of systemic metabolic acidosis after cardiac arrest may improve neurological outcome. Because substantial controversy exists regarding whether systemic treatment with sodium bicarbonate is beneficial in cardiopulmonary resuscitation, the current model, in conjunction with the <sup>31</sup>P NMR measurement of pH<sub>i</sub>, provides a means to study the mechanism of postischemic normalization of intracellular and extracellular pH and to evaluate the treatment along with the long-term outcome.

The no-reflow phenomenon in the brain is believed to be one of the limiting factors for postischemic survival.<sup>14,42</sup>

Reflow-promoting therapy has been suggested to prevent injury after prolonged circulatory arrest.<sup>14</sup> Compared with the earlier resuscitation model with extrathoracic compression,<sup>33</sup> the relatively better outcome seen in the present model may result from the initial arterial blood infusion into the thoracic aorta; the high perfusion pressure helped to overcome the resistance in the microvasculature due to increased blood viscosity and reduced microvascular lumen. Indeed, the initial hyperperfusion during blood-infusion resuscitation can be seen clearly in the studies of MR cerebral perfusion mapping with this model. This aspect, and particularly the MR quantification of the protracted cerebral hypoperfusion at later stage of reperfusion, will be presented elsewhere.

In conclusion, an outcome model of cardiac arrest and resuscitation in rats is presented. The model is ideally suited for investigations in which remote resuscitation with minimal disturbance to animal position is required. Examination of injury development and maturation, evaluation of treatment strategies, and follow-up of long-term outcome are all possible with this model.

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## Editorial Comment

Complex, remote physiological manipulations of animals in an NMR spectrometer are not new to Dr Xu, the senior author. He previously used NMR to study brain intracellular pH in very hypercapnic rats ( $\text{Pco}_2 \approx 750$  mm Hg) in a hyperbaric chamber.<sup>1</sup> Although advantages of rodent models include reduced animal costs, relevant cerebrovasculature, and a small brain size convenient for fixation and immunohistochemistry, the impressive new “in-the-magnet” stroke model of Liachenko et al is clearly “technology driven.” Rodents fit into high-field magnets, and the new model opens rodent studies of complete cerebral ischemia to sophisticated NMR methodologies currently being used for preclinical studies of focal cerebral ischemia. The article is appropriately brief about plans for future rodent studies. It stays focused on details of the model. However, we must appreciate that the authors have a high-field system (9.4-T magnet; 400-MHz proton frequency), with custom-made radiofrequency coils and a versatile computer console. From an NMR perspective, sophisticated NMR spectroscopy and MRI techniques are possible, and also improved, by having smaller animals at higher magnetic fields. For example, spin tagging of arterial water protons in the neck should allow for reliable perfusion MRI images (ie, images of cerebral blood flow) in the rodents. Tagged water protons can traverse the brain more quickly in the rat than in the human, where transit time, long enough for relaxation effects to be important, is a limiting factor for NMR approaches to cerebral blood flow determinations.<sup>2</sup> Similarly, brain imaging of pH and lactate,<sup>3,4</sup> as well as several other NMR methods, should be more feasible at high magnetic fields. Ultimately, technological NMR advances might permit in vivo physiological monitoring to be accomplished completely via specialized NMR imaging and spectroscopy. NMR-derived parameters can be used to regulate and define physiological insults, while neurological examinations and histology/immunohistochemistry are used to quantify outcome and injury. Such physiological monitoring would, of course, be even more welcome for studies in mice, where it could assist valuable testing of genetic modulations. But even if NMR methods are not extending rapidly to mice, genetic methods are slowly being extended to rats. Genetic engineering in rats has become a reality, with genetic overexpression being accomplished more easily at this time

than genetic knock-out.<sup>5</sup> One can imagine, for example, using the authors’ model to study transgenic rats that overexpress human copper-zinc superoxide dismutase 1.<sup>5</sup> The authors could test interesting hypotheses about the role of oxidative stress<sup>6</sup> in complete cerebral ischemia.

But what about the fact that the authors’ model employs asphyxia? Other “in-the-magnet” cardiac arrest models with larger animals have been used for important studies,<sup>7,8</sup> but with fibrillation followed by defibrillation. Such an approach more closely models the situation for adult humans. Asphyxial cardiac arrest is more relevant to pediatric global ischemia, a point appreciated and discussed by the authors. If asphyxia is a disadvantage to the model, a redeeming aspect is the consistency and reproducibility of hemodynamic and metabolic resuscitation. Perhaps the model’s achievements in resuscitation exploit the fact that fibrillating rodent hearts can be defibrillated without electroshock.

When investigators undertake in-the-magnet studies, will they make this model as popular as the “suture occlusion” model<sup>9–11</sup> has been made for NMR studies of focal ischemia<sup>12,13</sup>? As stated in a study and review by Laing et al,<sup>14</sup> the “suture occlusion” model was first published in 1986 by Koizumi et al<sup>9</sup> and then published in a variation by Longa et al.<sup>10</sup> Important information about the evolution of T2-weighted imaging and diffusion-weighted imaging in focal ischemia, first done in animal studies,<sup>15</sup> is turning out to be very useful in MRI evaluations of human stroke,<sup>16</sup> especially in differentiating new infarcts from old ones. In summary, the new complete cerebral ischemia model by Liachenko et al should permit important rodent studies of new drugs and regimens. If protocols can be found that improve rodent neurological outcome after cardiac arrest, one can then search for NMR/MRI parameters that are associated with improvement and gain insight into mechanisms and measures to be focused upon in humans. Interest in the authors’ stroke model will clearly increase after it is used for important preclinical NMR investigations.

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