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# An Experimental Circulatory Arrest Model in the Rat to Evaluate Calcium Antagonists in Cerebral Resuscitation

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## ABSTRACT

A circulatory arrest model in the rat was developed for use in cerebral and cardiac resuscitation studies. Whole-body ischemia was produced for 8 to 18 minutes by arresting the heart with a cold potassium chloride cardioplegic solution. Following cardiopulmonary resuscitation, minimal, standardized intensive care was provided. As the duration of ischemia was increased from 8 to 18 minutes, survival immediately following resuscitation decreased from 100% to 25%, and survival at 48 hours after ischemia decreased from 80% to 0%. Thirty per cent of the rats recovering from 11 minutes of ischemia suffered motor seizures. Survival and the incidence of motor seizures appear to be good measures of outcome following ischemic circulatory arrest. These measures can be used to test the possible anti-ischemic actions of calcium antagonists or other drugs.

**Key words:** animal model, calcium antagonists, cardiac arrest, cerebral ischemia, CPR, long term survival, potassium-induced arrest

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## **INTRODUCTION**

Between 10% and 40% of people who survive out of hospital circulatory arrest experience significant neurological damage [1]. Some of the damage caused by ischemic anoxia occurs after reperfusion and is amenable to therapy [2]. Intense effort is under way to discover drug therapies that attenuate postischemic encephalopathy, even though the drugs would be given after the period of ischemia--that is, after cardiopulmonary resuscitation (CPR) and restoration of circulation. Such research requires efficient, reproducible, and, if possible, low cost animal models.

Animal models previously described in cerebral resuscitation studies employ dogs or monkeys as subjects [3-5]. The canine model developed by Safar et al. [3] is created by inducing 12 minutes of unsupported ventricular fibrillation, from which as many as 67% (23/ 34) of the dogs are never resuscitated and therefore do not enter into the cerebral resuscitation phase of the study. Bleyaert and co-workers [5] have developed a high-pressure neck tourniquet model in the monkey. In this model, whole-body ischemia was avoided in order to minimize problems with extracranial complications. Still, 25 of 62 animals could not be included in the final results, owing to various experimental difficulties. Such loss of expensive laboratory animals is inefficient and costly, and has encouraged several groups to consider small animal models. In this study we present the development and characterization of a circulatory arrest model in the rat, which can be used to evaluate the effects of cerebral resuscitation measures such as treatment with calcium antagonists or barbiturates.

## **MATERIALS AND METHODS**

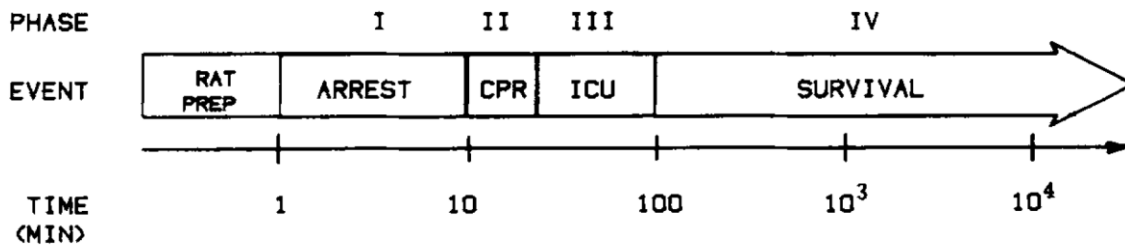
### **Rat Circulatory Arrest Model**

Male Wistar rats were used in all studies. Before experimentation, they were housed in a 12-hour light-dark cycle that started at 6:00 AM. Food and water were provided ad libitum.

Ketamine was used as the anesthetic agent because it has lesser effects on the cardiovascular system and plasma catecholamine levels than other agents [6] and because, unlike barbiturates [7], it is not thought to block membrane calcium channels. Each rat was ventilated by use of pressure-limited jet ventilation via a 16-gauge Teflon<sup>®</sup> cannula inserted through a midline tracheostomy. Tidal volume during artificial ventilation ranged from 2 to 5 ml. A 2-mm diameter, soft rubber stomach tube was placed to release trapped gases from the stomach. Succinylcholine (1.5 mg/kg) was administered intraperitoneally 5 minutes before the arrest to block gasping, which in itself can create blood flow during cardiac arrest [8]. A heat lamp was used to maintain rectal temperature between 36 and 38 °C during the prearrest and postarrest phases. During the circulatory arrest the rat was allowed to cool, as would usually occur in clinical cardiac arrest.

The four major phases of the protocol are illustrated in Figure 1. They are the arrest, resuscitation, intensive care, and survival phases. The specific steps taken in each phase are listed in Table 1. In phase I, cardiopulmonary arrest was accomplished in 94 rats ranging in body weight from 350 to 450 grams by cessation of jet ventilation and injection of 0.4 to 0.8 ml of a cold 1% KCl cardioplegic solution percutaneously into the left ventricle of the heart.

Initially, a 0.4 ml bolus of KCl was injected rapidly into the left ventricle of the heart. If within the first 30 seconds of the arrest an apex beat was detected, additional 0.2-ml volumes of KCl were injected, up to a total of 0.8 ml. In approximately half the animals, a weak apex beat returned after KCl injection and cessation of ventilation, and gentle thoracic pressure (“chest restriction”) was applied transthoracically with two fingers at the level of the heart. Such chest restriction caused disappearance of pulses within a few seconds and aborted spontaneous recovery from the arrest. Arrest was verified by the absence of a palpable apex beat. The arrest was allowed to continue for a predetermined number of minutes, after which time attempts were made to resuscitate the rats.



**FIGURE 1.** The four major phases of the rat circulatory arrest model. The preparatory phase is followed by phase I, potassium-induced cardiac arrest; phase II, cardiopulmonary resuscitation; phase III, minimal intensive care; and phase IV, survival and qualitative assessment of neurological outcome. The time line shows approximate times of each phase.

**TABLE 1. Procedure for Cardiac Arrest in Rats**

Phase	Time (min)	Events
prep	- 15	160 mg/kg ketamine, intraperitoneally, ECG (lead II), palpate apex beat
prep	- 10	Midline tracheostomy, intubate trachea and stomach, begin jet ventilation: 40/min, 20–30 cm H <sub>2</sub> O
prep	- 5	1.5 mg/kg succinylcholine, intraperitoneally
I	0	Cardiopulmonary arrest: cease jet ventilation, inject 0.4–0.8 ml 1% KCl 4°C solution percutaneously into left ventricle
I	0–3	Chest restriction
II	10	Rat IAC-CPR, resume ventilation: 70/min, 40–60 cm H <sub>2</sub> O
II	12*	Restoration of apex beat
II	17*	Ventilation rate reduced to 40/min, 20–30 cm H <sub>2</sub> O
III	30*	Rat moved to intensive care, 5 ml 5% dextrose in water and 5 ml lactated Ringer's solution, subcutaneously, continued ventilation: 40/min, 20–30 cm H <sub>2</sub> O, 37°C heating pad
III	60*	Spontaneous respirations begin, tracheal tube removed, neck wound sutured, and rat returned to its cage
IV	48 hr	Monitor survival and seizure activity

\*These times are approximate and may differ with each rat.

Interposed abdominal compression-CPR (IAC-CPR) [9] and jet ventilation (frequency 70/min) were used to resuscitate the rats. The IAC-CPR was performed as follows: two fingers of one hand were placed midline on the sternum halfway between the xyphoid and the manubrium so that the heart was directly compressed, and three fingers of the other hand were placed on the lower abdomen to perform the abdominal compressions. Thoracic compressions were simultaneous with expiration, and abdominal compressions were interposed between thoracic compressions. Palpation of an apex beat was used to establish return of the circulation. The duration of ischemia was measured from the onset of the sudden drop in blood pressure or the loss of an apex beat to the restoration of a pulse pressure or apex beat.

In phase III, minimal and uniform intensive care was supplied to each rat. Approximately 5 minutes after resuscitation, jet ventilation was reduced to 40/min. Thirty minutes after ischemia, 5 ml each of a 5% dextrose solution and a lactated Ringer's solution were injected subcutaneously to maintain hydration. The rats were placed on a 37 °C heating pad, and artificial ventilation was continued at a rate of 40/min until vigorous spontaneous respirations were observed. At this time the tracheal tubes were removed from the rats, their neck wounds sutured, and the animals returned to individual cages. In phase IV, survival and qualitative neurological outcome were monitored.

### **Confirmation of Circulation Arrest**

To establish that palpation of the apex beat transcutaneously was a reliable method of monitoring cardiac mechanical activity, we conducted a study in 15 rats to verify circulatory arrest. The animals underwent arrest with KCl injection as described, and carotid arterial blood pressure was monitored with a disposable pressure transducer (Cobe Laboratories, Inc., Lakewood, Colorado). The presence or absence of an apex beat as determined by the experimenter was compared in time to arterial pressure recordings. In the subsequent survival study, cardiac arrest was confirmed only by disappearance of the palpable apex beat to minimize the amount of surgical trauma and to avoid ligating a carotid artery.

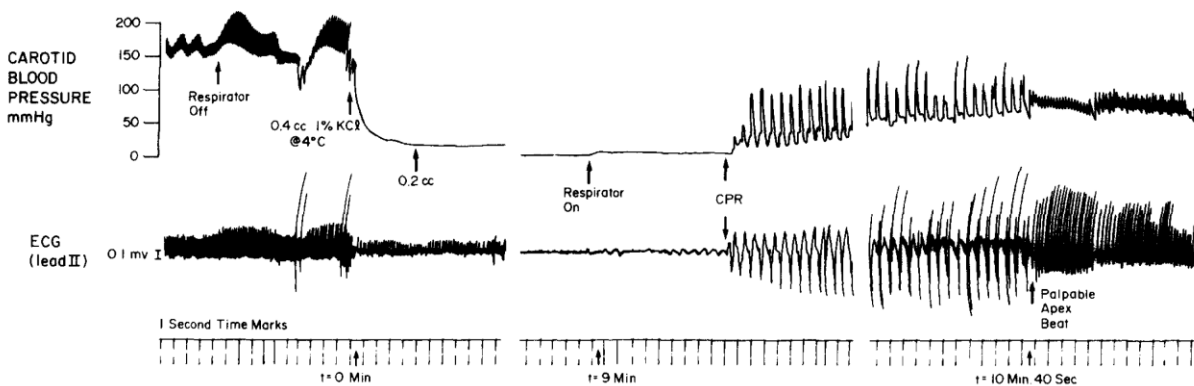
### **Characterization of the Model: Survival Study**

To characterize this rat circulatory arrest model, a survival study was conducted without drug intervention. In this study 79 rats were subjected to an ischemic insult (6 to 12 minutes of total circulatory arrest followed by IAC-CPR as previously described), and survival was monitored for 48 hours after ischemia.

## RESULTS

### Confirmation of Circulatory Arrest

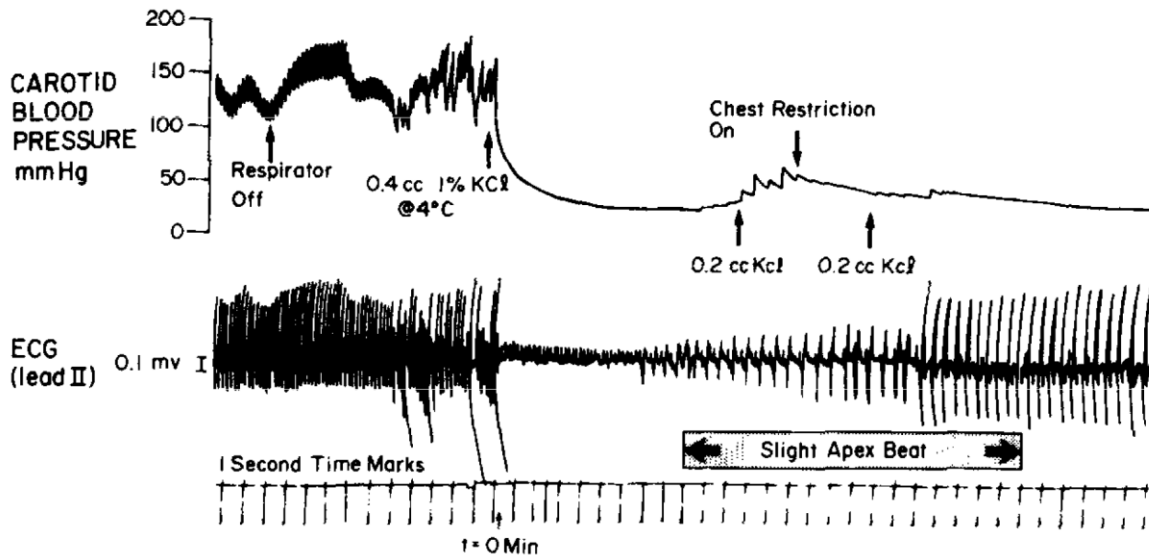
Illustrated in Figure 2 is a typical record of one of the 15 rats in which blood pressure was measured to verify circulatory arrest. Immediately after KCl injection, arterial blood pressure fell promptly to about 20 mm Hg without pulsations. Injection of KCl produced an electromechanical dissociation. After 9 minutes of circulatory arrest, IAC-CPR was begun. In this rat the circulation returned spontaneously after 1 minute, 40 seconds of IAC-CPR. The palpable apex beat was correlated in time to the graphic record, showing recovery of pulsatile blood pressure as an indicator of circulatory arrest.



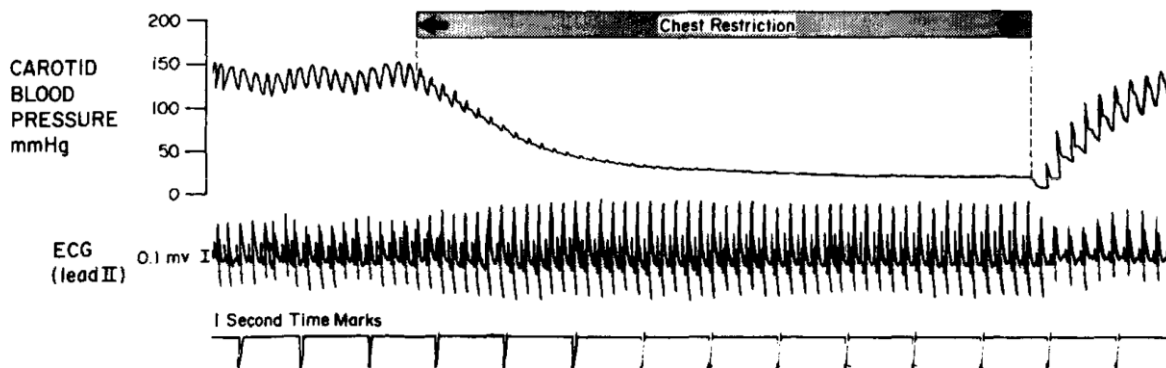
**FIGURE 2.** Physiograph record of the arrest and resuscitation of a rat. Channels 1 and 2 illustrate carotid blood pressure and electrocardiogram, respectively. At  $t = 0$  min, 0.4 ml cold KCl was injected percutaneously into the left ventricle of the heart, causing a rapid drop in blood pressure. An additional 0.2 ml was injected to ensure continued arrest of the heart. At  $t = 9$  min, artificial ventilation was resumed and a series of deep ventilations (sighs) were given before cardiopulmonary resuscitation (CPR) was begun. The pulsatile pressure waveform and elevated diastolic pressure illustrate the effectiveness of IAC-CPR in rats. Approximately 1 minute, 40 seconds of CPR elapsed before the rat's heart spontaneously recovered and a palpable apex beat was detected. Palpation of the apex beat correlated well in time with the presence of arterial pressure pulses in the carotid artery.

In none of the 15 rats was a palpable apex beat detected when arterial pressure pulses were absent. We did not detect an apex beat by palpation when pressure pulses of 10 mm Hg were present in a single rat. In all the 15 rats in which we monitored carotid blood pressure, we were able to abruptly arrest the heart as indicated by a drop in arterial blood pressure to 20 mm Hg or less. Within the first 2 minutes of arrest there were sporadic pressure pulses in eight of the 15 rats, but these were no greater than 20 mm Hg in amplitude. As shown in Figure 3, chest restriction and additional doses of KCl aborted the spontaneous recovery of the arrested heart.

The effectiveness of chest restriction alone in preventing cardiac pumping in the rat is clearly illustrated in Figure 4. The combination of KCl injection and chest restriction successfully produced controlled circulatory arrest in 15 of 15 rats. Having completed this preliminary study in the 15 rats, we felt confident in monitoring cardiac mechanical activity solely by palpation of the apex beat.



**FIGURE 3.** Spontaneous recovery of a rat heart shortly after the initial arrest. Additional ECG doses of KCl were injected, and chest restriction was applied. The combination of these procedures effectively abolished the arterial pressure pulses and apex beat.



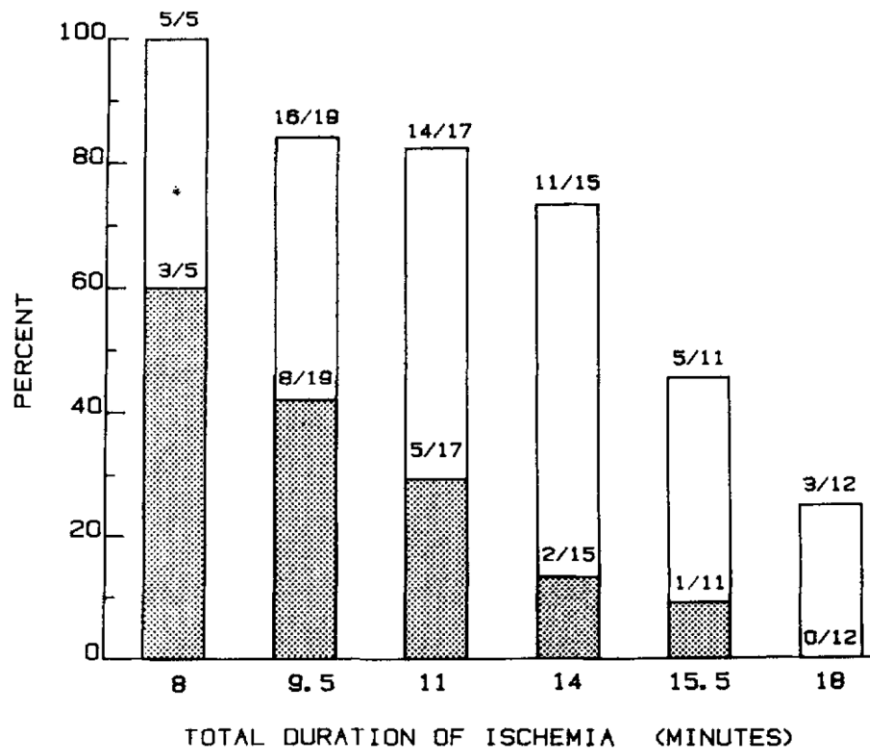
**FIGURE 4.** The effect of chest restriction on arterial blood pressure in a normally beating rat heart. Approximately 20 mm Hg of pressure was applied transthoracically with thumb and index finger at the level of the heart, causing arterial blood pressure to fall to a minimum of 25 mm Hg. The highly compliant rat chest and small size of the rat probably account for the effectiveness of chest restriction.



## Survival

For all the durations of ischemia tested, 33% (26/79) of the rats were not resuscitated. Of those rats not resuscitated, 50% (13/26) were observed to have thoracic damage upon gross postmortem examination because of experimental difficulties.

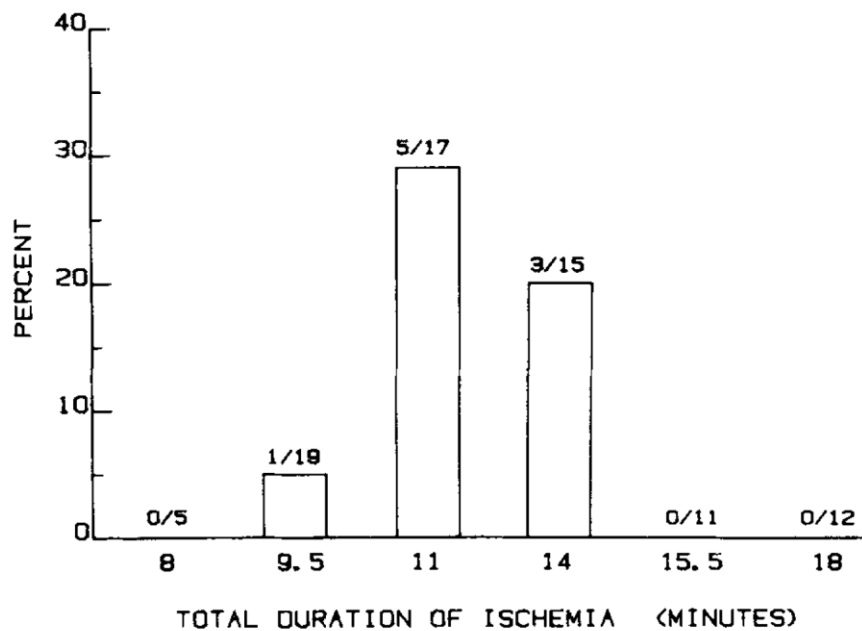
The results of the survival study are recorded as percentage of rats resuscitated and percentage of the total rats alive 48 hours after ischemia (Fig. 5). The results are presented with respect to the total duration of ischemia, which is defined as the time to definitive therapy and is equal to the arrest time plus the CPR time. After 8 minutes of ischemia, a pulse was restored in 100% of the rats, and 60% of all rats were alive 48 hours after ischemia. After 18 minutes of ischemia, 25% of rats were resuscitated and 0% were alive at 48 hours. For the intermediate durations of ischemia, the resuscitation and survival rates decreased linearly. The resuscitation and survival rates were statistically different ( $P \leq 0.05$ ) between the 8-minute and 18-minute durations of ischemia, according to Fisher's exact test. Although statistical significance was not observed between any of the other groups, the trend is evident.



**FIGURE 5.** Percentage of resuscitation and survival as a function of the total duration of ischemia. Open bars represent the percentage of rats resuscitated, as demonstrated by restoration of pulse. Shaded bars represent the percentage of rats in each group that were alive 48 hours after ischemia. The ratios above each bar reflect the absolute numbers of rats in the respective groups.

## Motor Seizures

This ischemia rat model produced high incidence of motor seizures following arrest and resuscitation. These seizures were characterized by the rat's running rapidly in the cage for approximately 1/2 to 2 minutes. Seizures would end when the rat collapsed into a flaccid state suggestive of postictal depression. In some cases presumably more severe, the seizures progressed into a tonic-clonic stage characterized by opisthotonus. These animals also showed signs of severe postictal depression. The number of rats suffering seizures for each duration of ischemia is shown in Figure 6. At 8, 15.5, and 18 minutes of ischemia there were no seizures. The greatest rate of seizures (30%) was produced by the 11-minute duration of ischemia.



**FIGURE 6.** Percentage of total number of rats at each duration of ischemia having motor seizure activity in the 48 hours after ischemia. No rats had seizures at the extreme durations of ischemia, but a maximum of 30% (5/17) of the rats surviving 11 minutes of ischemia had seizures.

## DISCUSSION

In the survival study presented here, we have measured the resuscitation, survival rates, and occurrence of seizure activity with whole-body ischemia ranging from 8 to 18 minutes. The results suggest that an efficient duration of ischemia for drug intervention studies would be 11 minutes. At this duration there was a resuscitation rate of at least 80% and approximately 40% survival 48 hours after ischemia. The high resuscitation rate makes the model efficient, since only one in five rats failed to enter into the cerebral resuscitation phase of the study. With a 40%

survival rate at 48 hours, it should be possible to detect changes in survival and seizure activity in drug-treated rats compared with controls.

The previously unreported observation of motor seizures in resuscitated rats has proved to be a simple, objective measure of cerebral dysfunction. We believe, as does Edmeads [10] that three cell populations exist in the brain following an ischemia insult: 1) healthy neurons and neuroglia that survive the insult unharmed, 2) cells that die outright from the insult, and 3) cells that are damaged and may either survive or die depending on the course of therapy. These damaged cells of the brain are the focus of aggressive cerebral resuscitation efforts. The damaged neurons probably function as foci of the CNS seizure activity. Thus, the seizure activity we have observed probably reflects the presence of damaged neurons in the brain. Accordingly, interventions that both improve survival and diminish seizure activity in this model hold promise for clinical application in attenuation of postischemic encephalopathy.

All animal and human studies of brain resuscitation face four fundamental problems that make interpretation of results difficult:

First, complete cerebral ischemia must be verified. Neck tourniquet models do not generally occlude the vertebral arteries, protected in their bony canals. Models requiring surgical ligation of the carotid and vertebral arteries are at the mercy of variable degrees of collateral circulation via the spinal and subclavian arteries. In models such as the present one using cardioplegia solution, the heart may escape control and begin beating normally. Only documented ventricular fibrillation produces certain circulatory arrest, but this arrhythmia is not reliably sustained in small animals [11].

Second, cardiac resuscitation from whole-body ischemia severe enough to create significant brain dysfunction is extremely difficult. Hendrickx et al. [12] have developed a rat circulatory arrest model in which they create total circulatory arrest by asphyxiation. In this model, cardiac mechanical activity persists during a portion of the asphyxia time. Hermans and co workers [11] create circulatory arrest by such rapid cardiac electrical stimulation that the rats are in a state of pseudo-fibrillation. There are major differences from our model and theirs. Normothermic cardiac ischemia with fibrillation or continuous cardiac mechanical activity (as in the early stages of asphyxia) results in greater myocardial oxygen demand and debt than in a hypothermic, potassium-arrested heart [13, 14]. High-energy phosphate levels in the myocardium of isolated rat hearts were determined to be at least twice as great in a normothermic potassium-arrested heart than in a normothermic fibrillating heart [15]. Myocardial ischemia following either asphyxia or fibrillation may limit the duration of whole-body ischemia from which the rat can be resuscitated. The duration of insult may therefore be too short to create significant cerebral damage. We arrest the heart with a cold potassium cardioplegic solution and can achieve survival at longer durations of whole-body ischemia, thereby producing significant cerebral dysfunction. This provides a suitable pathological substrate for testing therapeutic interventions.

Third, outcome is often influenced by extracranial complications, such as pulmonary edema, sepsis, and cardiogenic shock. Each of these conditions can produce deterioration of neurological status that is unrelated to both the primary ischemic-anoxic event and the experimental therapy directed toward it. Such extracranial complications have produced major experimental

difficulties in the monkey model of Bleyaert et al. [5] and in the studies of White et al. [4] as well, requiring extensive staffing and expense to keep the animals alive. Extracranial complications do not appear to be a major complication in our model. Pulmonary edema and sepsis were not seen with regularity in the arrested rats we studied. Since arrest can be monitored by palpation, only a single incision is required for the tracheal cannula, and this has not been the site of complications. (In early studies to show circulatory arrest with an intravascular cannula, leg edema and gangrene did follow femoral artery catheterization.)

Fourth, intensive care procedures vary during the postischemic phase. To survive to a stage of stable neurological deficit, large animals and human beings require more elaborate intensive care, including mechanical ventilation, fluids, pressor drugs, antibiotics, and inotropic agents. If these therapies are individualized, as in clinical practice, experimental and control subjects are not strictly comparable, and the validity of the study is compromised. Intensive care measures in this model are deliberately kept to a minimum and are invariant.

## **CONCLUSION**

We believe this model provides satisfactory circulatory arrest for cerebral resuscitation studies. Although the ventricles sometimes escape from the influence of intracardiac KCl, the addition of gentle chest restriction appears to abort the return of circulation by limiting cardiac filling and/or emptying in small animals, such as rats, with compliant chest walls. In this way, circulatory arrest is produced reliably without surgery. We also believe that the proposed circulatory arrest model is simple, easy to replicate in other laboratories, and capable of generating statistically significant survival data with large numbers of subjects at reasonable cost and effort. Moreover, this method could be useful in screening the large numbers of compounds potentially beneficial in ameliorating permanent disability following cardiac arrest and CPR.

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## **REFERENCES**

1. Liberthson R, Nagel E, Hirschmann J. Prehospital ventricular defibrillation: Prognosis and followup course. *N Engl J Med* 1974;291:317-321.
2. White BC, Winegar CD, Wilson RF, et al. Possible role of calcium blockers in cerebral resuscitation: A review of the literature and synthesis for future studies. *Crit Care Med* 1983;11:202-207.
3. Safar P, Stezoski W, Nemoto EM. Amelioration of brain damage after 12 minutes' cardiac arrest in dogs. *Arch Neurol* 1976;33:91-95.
4. White BC, Winegar CD, Jackson RE, et al. Cerebral cortical perfusion during and following resuscitation from cardiac arrest in dogs. *Am J Emerg Med* 1983;1:128-138.

5. Bleyaert A, Safar P, Nemoto E, et al. Effect of postcirculatory-arrest life-support on neurological recovery in monkeys. *Crit Care Med* 1980;8:153-156.
6. Chernow B, Lake CR, Cruess D, et al. Plasma, urine, and CSF catecholamine concentrations during and after ketamine anesthesia. *Crit Care Med* 1982;10:600-603.
7. Altura BM, Altura BT. Pharmacologic inhibition of cerebral vasospasm in ischemia, hallucinogen ingestion, and hypomagnesemia: Barbiturates, calcium antagonists, and magnesium. *Am J Emerg Med* 1983;2:180-190.
8. Bircher N, Safar P, Eshel G, et al. Cerebral and hemodynamic variables during cough-induced CPR in dogs. *Crit Care Med* 1982;10:104-107.
9. Ralston SH, Babbs CF, Niebauer MJ. Cardiopulmonary resuscitation with interposed abdominal compression in dogs. *Anesth Analg* 1982;61:645-651.
10. Edmeads J. Bringing the brain back alive. *Emerg Med* 1982;14:111-119.
11. Hermans C, De Reese R, Van Loots J, et al. A cardiac arrest model in rats for evaluating the antihypoxic action of flunarizine. *Eur J Pharmacol* 1982;81:137-140.
12. Hendrickx HHL, Gisvold SE, Safar P, et al. A new cardiac arrest rat model for brain resuscitation studies. *J Cereb Blood Flow Metab* 1981 ;l(suppl 1):S222-S223.
13. Buckberg GD, Brazier JR, Nelson RL, et al. Studies of the effects of hypothermia on regional myocardial blood flow and metabolism during cardiopulmonary bypass: I. The adequately perfused beating, fibrillating, and arrested heart. *J Thorac Cardiovasc Surg* 1977;73:87-94.
14. McConnell DH, Brazier JR, Cooper N, et al. Studies of the effects of hypothermia on regional myocardial blood flow and metabolism during cardiopulmonary bypass: II. Ischemia during moderate hypothermia in continually perfused beating hearts. *J Thorac Cardiovasc Surg* 1977;73:95-101.
15. Hearse DJ, Stewart DA, Chain EB. Recovery from cardiac bypass and elective cardiac arrest: The metabolic consequences of various cardioplegic procedures in the isolated rat heart. *Circ Res* 1974;35:448-457.