

# Animal Models of Focal and Global Cerebral Ischemia

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## Abstract

The use of appropriate animal models is essential to predict the value and effect of therapeutic approaches in human subjects. Focal (stroke) and global (cardiac arrest) cerebral ischemia represents diseases that are common in the human population. Stroke and cardiac arrest, which are major causes of death and disability, affect millions of individuals around the world and are responsible for the leading health care costs of all diseases. Understanding the mechanisms of injury and neuroprotection in these diseases is critical if we are ever to learn new target sites to treat ischemia. There are many animal models available to investigate injury mechanisms and neuroprotective strategies. This review summarizes many (but not all) small and large animal models of focal and global cerebral ischemia and discusses their advantages and disadvantages.

**Key Words:** animal models; cardiac arrest; focal and global cerebral ischemia; stroke

## Introduction

The goal of cerebral ischemia (focal and global) models is to reduce oxygen and glucose supply to brain tissue. This process produces brain injury via a variety of cellular and molecular mechanisms that impair the energetics required to maintain ionic gradients. The mechanisms involve a complex series of pathophysiological events that are dependent on the severity, duration, and location of the ischemia within the brain. A simple overview (Figure 1) of these pathophysiological mechanisms is that energy failure results in neuron depolarization, which causes activation of glutamate receptors, which in turn alters ionic gradients of  $\text{Na}^+$ ,  $\text{Ca}^{++}$ ,  $\text{Cl}^-$ , and  $\text{K}^+$ . As glutamate increases in the extracellular space, peri-infarct depolarization occurs. Then, as water shifts occur, cells swell with resulting cerebral edema. The result of increasing intracellular  $\text{Ca}^{++}$  is an up-regulation of a variety of enzyme systems such as lipases, proteases, and endonucleases. As a result, free  $\text{O}_2$  radicals are generated via a variety of biochemical pathways, and

apoptotic cell death occurs. Free radicals also induce formation of a variety of inflammatory mediators such as platelet and endothelium selectins, a variety of molecules, platelet activating factor, tumor necrosis factor  $\alpha$ , and an assortment of interleukins. It is critical to comprehend these complicated pathophysiological cascades of molecular and cellular events resulting from ischemia completely because one of the goals of utilizing animal models of cerebral ischemia is to dissect apart these various mechanisms of injury to arrive at a potential target site for treatment of ischemic injury (i.e., neuroprotection). An excellent review of these cascades is provided by Dirnagl et al. (1999).

Volumes have been written since the 1960s concerning many potential neuroprotective agents using animal models. Many of these preclinical studies have demonstrated neuroprotective effects of many pharmacological agents in a variety of ischemic models and in a variety of species. However, when these pharmacological agents have been taken to human trials, they have been resoundingly unsuccessful. The reasons for this failure are many and complex, and they may involve timing of the administration of the drug, window of opportunity, length of time of ischemia, dose of drug given, species, gender differences, age, and underlying diseases. Another issue involves clinical trial design. In spite of potentially effective treatments in animal models, many stroke trials have failed due to naive clinical trial design (i.e., wrong selection criteria for heterogeneous stroke patients, wrong outcome measures, or wrong time window and dose administration of the drug).

We must also consider that the animal models we utilize for preclinical testing do not reflect the disease as it occurs in humans. This limitation may account for why there are so many different models of cerebral ischemia in use today. Are the models that have been developed the best models to study research aspects involving mechanisms of injury of disease, and neuroprotection; or are these models simply not reflective of mechanisms of injury and neuroprotection as they pertain to the human disease situation?

## Size of Animals as Models

Models of cerebral ischemia can be performed in both small and large animals (e.g., mice, rats, gerbils, rabbits, cats, dogs, pigs, sheep, and monkeys). Advantages and disadvantages of different sizes of animals are discussed below.

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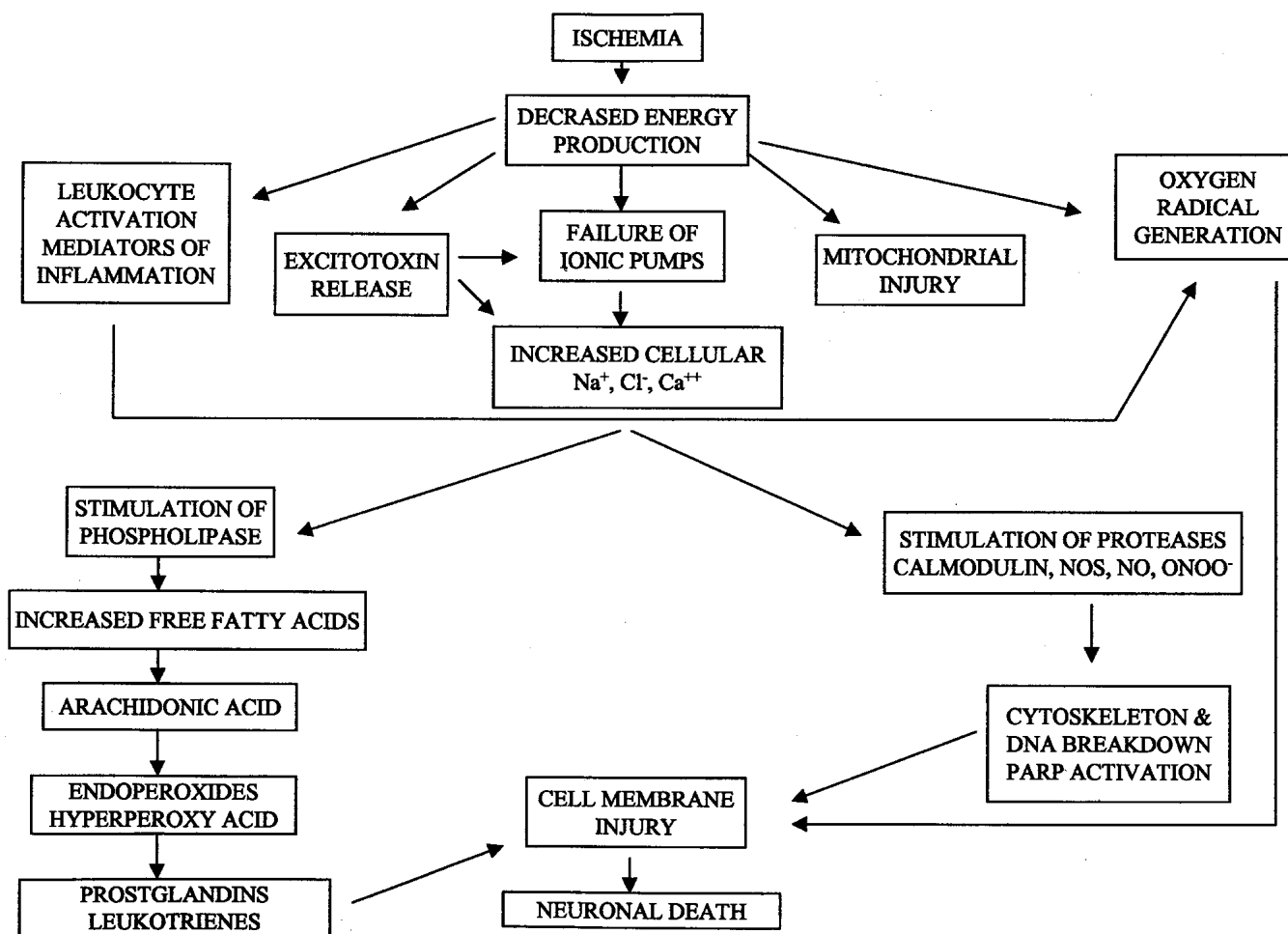


Figure 1 Potential mechanisms of injury from ischemia.

## Large Animal Models

There are many advantages of utilizing large animals to study cerebral ischemic effects. The relatively new regional imaging techniques, specifically nuclear magnetic resonance spectroscopy, imaging, and functional imaging, have traditionally been easier to perform on large animals (although these sophisticated techniques have recently begun to be applied also to smaller animals such as mice and rats [Klein and Nelson 2002; Landi 2001]). In larger animals, it is easier to perform sophisticated physiological monitoring (e.g., evoked potential monitoring, electroencephalography, arterial blood gases, blood pressure, blood glucose, lactate, and hemoglobin measurements). All of these measurements can be made simultaneously, at multiple time points, in the same animal. The measurements can be coupled with neurological examination, neurobehavior, neurochemistry, and neuropathology, also performed in the same animal. In addition, measurements of regional cerebral blood flow and metabolism can be made easily in large animal models. Finally, the brains of large animals are gyrencephalic, like humans, which may make them closer to the human brain in structure and function.

Nevertheless, there are several disadvantages of utilizing large animals, which involve the use of invasive surgery for monitoring and for producing ischemia. Many times in using focal ischemia models, the dura is opened. In large animals, there is great injury or infarct size variability and physiological variability, in addition to the significant mortality of acute and chronic survival models. These large animals models are also financially very costly and labor intensive, and the completeness of occlusion (or ischemia) may be unclear. Using these large animals may also necessitate the use of completely different anesthetic regimens, which may modify outcome from ischemia. Finally, there are public animal welfare concerns regarding the use of large animals (particularly dogs, cats, and monkeys).

## Small Animal Models

In contrast to the many disadvantages of using large animals for research studies, the use of small animals presents clear advantages. Small animals (especially rodents) are much less costly to obtain and maintain for longer periods of time, and their supply costs are much less than for large animals.

Small animals (particularly mice) are genetically homogeneous, and genetic modifications can be made relatively easily to reproduce many different transgenic mice. With transgenic technologies, it is routine to overexpress certain genes, proteins, or enzymes in certain species, or to inactivate particular genes using a knockout approach; and this technology provides an excellent approach to help determine potential mechanisms of injury and neuroprotection. These approaches have been used most often in the mouse, although larger, more costly and labor-intensive species can be utilized. In addition, there is less concern over the use of rats and mice than of dogs, cats, or monkeys. Another considerable advantage in small animals, particularly mice and rats, is the ability to utilize sophisticated neurosensory and motor behavior measurements as outcome measures of injury from ischemia. The small brain size in mice and rats may be considered an advantage because it allows for certain fixation procedures such as *in vivo* quick freeze techniques for biochemical and neurochemical analysis (Ponten et al. 1973). These smaller animals, however, have lissencephalic brains and thus may be quite different in anatomy and functional aspects from human brain. Physiological monitoring is also considerably more challenging in small animals, and concurrent measurements over time are limited or may not be possible.

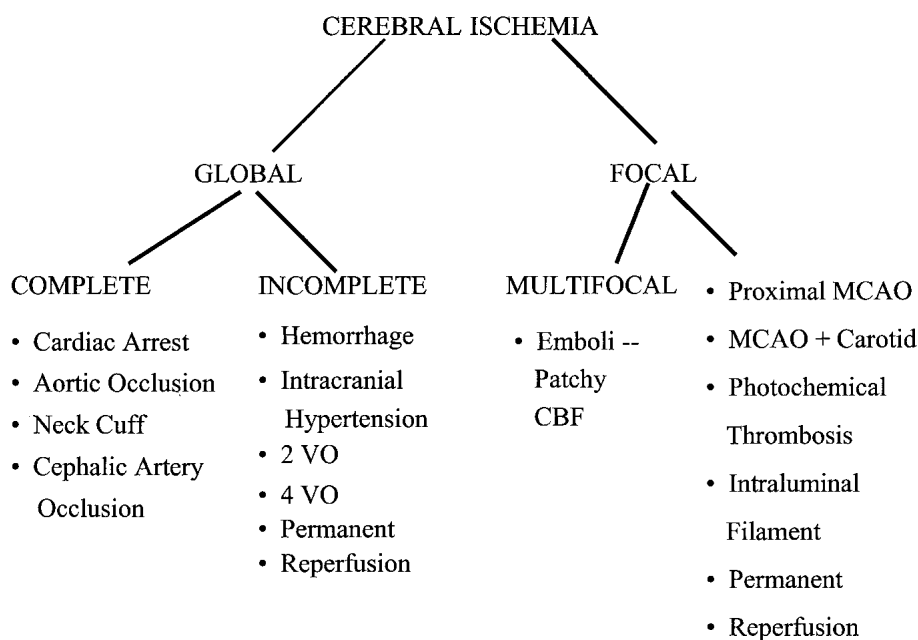
Despite the potential disadvantages of using animal models to study focal and global cerebral ischemia, these models will continue to be utilized (Ahmed et al. 2000; Ginsberg and Busto 1989, 1997; Koehler 1997). One clear aspect is that these models can be physiologically controlled so that the resulting injury is reproducible and variability is as limited as possible. These models also allow careful dis-

section of potential mechanisms of injury and neuroprotection and take into consideration timing of the events that occur from seconds, minutes, hours, and days after the ischemic event. All of these aspects are difficult, if not impossible, to determine and study during ischemia in humans because of the diversity of the human disease in terms of the causes, manifestations, and anatomic sites of the ischemia. In fact, this diversity in humans and coexisting diseases may be exactly why our animal models may not mimic human ischemic diseases but may instead be useful to study only mechanisms of injury and neuroprotection.

## Definitions of Focal and Global Cerebral Ischemia

Cerebral ischemia experimental models are characterized as global, focal, and multifocal ischemia (Figure 2). Global ischemia occurs when cerebral blood flow (CBF<sup>1</sup>) is reduced throughout most or all of the brain, whereas focal ischemia is represented by a reduction in blood flow to a very distinct, specific brain region. In multifocal ischemia, there is a patchy pattern of reduced CBF. With complete ischemia, global blood flow has ceased completely; whereas with incomplete ischemia, global blood flow is severely

<sup>1</sup>Abbreviations used in this article: CBF, cerebral blood flow; CPR, cardiopulmonary resuscitation; MCA, middle cerebral artery; MCAO, middle cerebral artery occlusion; SHR, spontaneous hypertensive rat; SHRSP, spontaneous hypertensive stroke-prone rat; 2-VO, two-vessel occlusion; 4-VO, four-vessel occlusion.



**Figure 2** Pathophysiological mechanisms involved in ischemia within the brain. VO, vessel occlusion; CBF, cerebral blood flow; MCAO, middle cerebral artery occlusion.

reduced but the amount of flow is insufficient to maintain cerebral metabolism and function. In focal cerebral ischemia, there may be absolutely no blood flow in the very central core of the ischemia, but usually there is some flow that reaches the area via collateral circulation. Thus, there is usually a gradient of blood flow from the inner core reaching out to the limits of the ischemic area.

## Focal Cerebral Ischemia Models

Most focal cerebral ischemia models involve occlusion of one major cerebral blood vessel such as the middle cerebral artery (MCA<sup>1</sup>) in small animals (Garcia 1984; Hossmann 1991; Ponten et al. 1973) or large animals (Bose et al. 1984; Symon 1975; Symon et al. 1974). MCA occlusion (MCAO<sup>1</sup>) results in a reduction of CBF in both the striatum and cortex, but the degree and distribution of blood flow reduction depends on the duration of MCAO, the site of occlusion along the MCA, and the amount of collateral blood flow into the MCA territory. Several different types of MCAO models exist, and for the most part they are either a permanent or temporary (reperfusion) occlusion with MCA occlusion at either the proximal or distal part of the vessel. These MCAO models have been used extensively because of their purported relevance to human thromboembolic stroke (Garcia 1984; Hossmann 1991; Takizawa and Hakim 1991). There is widespread use of the models in studying pharmacological neuroprotection and mechanisms of injury from ischemia, and for characterization of genes and proteins involved in stroke.

Tamura et al. (1981a) devised a permanent proximal MCAO in rats, which involved subtemporal craniotomy. This model results in an infarction of the cortex and caudoputamen areas. Bederson et al. (1986) modified the technique to demonstrate how the precise site and extent of MCAO could influence neurological and neuropathological outcome. These latter studies also demonstrated that the lenticulostriate arteries and small cortical branches must be isolated from the proximal and distal sources of collateral supply to achieve a consistent infarction. Using this permanent MCAO model, it was found that local CBF in cortical areas in which histological abnormalities occur was approximately 25 mL/100 g/min, and the region of ischemic damage corresponded to the area of marked reduction in CBF (Bolander et al. 1989; Robinson et al. 1984; Tamura et al. 1981b). In a very interesting study, Duverger and MacKenzie (1988), using the Tamura model, compared outcome from permanent MCAO in several different rat strains. In comparing the hemispheric infarct volume of Wistar, Sprague-Dawley, and Fischer-344 rats, along with spontaneously hypertensive rats (SHRs<sup>1</sup>) and SHR-stroke prone rats (SHRSPs<sup>1</sup>), they found that the infarct of SHR and SHRSP strains was approximately 1.5 times that of the Wistar strain and that it was more consistent and larger in Sprague-Dawley rats. Of the normotensive strains, the infarct was largest and most consistent in the Fischer-344 rats.

Another variation of this MCAO model is to occlude the MCA in conjunction with the occlusion of the ipsilateral common carotid artery (Chen et al. 1986). This model has been achieved using a more distal MCAO above the rhinal fissure (Robinson and Coyle 1980; Robinson et al. 1975), and it offers a more simple surgical technique to approach the MCA. The common carotid artery is temporarily occluded, and flow is allowed to return at some later point. This technique was accomplished in the Long-Evans rat strain, and it was necessary to occlude the common carotid artery to reduce CBF in the MCA territory into the ischemic range. In this model, brains demonstrate moderate-sized infarcts in the frontoparietal cortex, and the caudoputamen is spared. In comparing histopathology in several rat strains, Brint et al. (1988) used MCAO coupled with permanent common carotid artery occlusion and found that cortical infarct volume was variable and inconsistent in Wistar and Fischer-344 rats, but was no larger and was quite reproducible in SHRs.

A number of investigators (Mohamed et al. 1985; Takagi et al. 1995; Tamura et al. 1981a; Tyson et al. 1984) have used electrocoagulation of the MCA to produce ischemia. With electrocoagulation, as shown for previous models, the area of reduced CBF corresponds closely to the area of neuropathological injury. Finally, some investigators have used the photochemical MCAO model, which involves irradiation of several branches of the distal MCA with beams from an argon dye laser following intravenous administration of the photosensitizing dye rose bengal (see Watson 1997 for review). This model requires only a small craniotomy, and the dura remains intact. Temporary common carotid artery occlusion can be added to restrict collateral supply to the area of the MCAO. This model results in a consistent infarction in the frontoparietal neocortex while sparing deep structures, and the area of infarction is larger and more consistent in Sprague-Dawley than in Wistar rats (Markgraf et al. 1993). The disadvantage of the model is that the photochemical reaction can result in microvascular injury.

Other models of focal ischemia have been utilized and include blood clot embolization in rats (Kaneko et al. 1985; Kudo et al. 1982) or large animals (Clark et al. 1991; de Ley et al. 1989; Dutka et al. 1987; Meyer et al. 1962) in which homologous blood clot fragments are injected directly into the common carotid artery or into a carotid artery via a retrograde catheter placed in an external carotid artery. Although this model is relatively easy to study, its main disadvantage is that the location of infarction(s) is not consistent.

Several models for MCA reperfusion have been developed over the years. Shigeno et al. (1985) placed a simple snare ligature around the MCA and were able to obtain occlusion and reperfusion by pulling and releasing the snare. This technique allows for the study of reperfusion cell injury, but the technique is technically demanding. Primates and cats have been the most commonly used large animals for this technique. Instead of a suture, a neurosurgical clip can be utilized to occlude the MCA, and the clip can be

removed to accomplish reperfusion (Matsumiya et al. 1990, 1991; Nishikawa et al. 1994; Symon et al. 1974; Takahashi et al. 1995; Takeshima et al. 1992, 1994; Weinstein et al. 1986).

Because the baboon is quite a large animal model, many physiological parameters can be measured simultaneously and over time. Symon et al. (1975a) measured CBF 3 yr after MCAO in baboons and found that CBF remained reduced in much of the tissue around the infarct even though there was no neuropathology in some regions. CO<sub>2</sub> reactivity and cerebral autoregulation were also impaired in the regions surrounding the infarct (Symon et al. 1975a). More complete, sophisticated neurological examinations can also be performed in baboons, and it has been found that the neurological deficits associated with MCAO in baboons closely resemble those seen in humans (Symon et al. 1975b; Weinstein et al. 1986). Facial and upper arm muscle weakness occurs immediately after MCAO, and some recovery may occur over months.

Another simple, relatively noninvasive method to produce either permanent or transient MCAO in rodents is with the use of the intraluminal filament. This technique has been very popular since its inception in the late 1980s (Koizumi et al. 1986; Zea Longa et al. 1989) for studying mechanisms of both cellular injury and neuroprotection. It involves inserting a 4-0 nylon suture into the internal carotid artery of rats and then advancing the thread cranially to block the MCA. This thread can be passed and is usually advanced 17 to 20 mm from the origin of the internal carotid artery, thus occluding collateral circulation from the anterior communicating arteries (Zea Longa et al. 1989). A laser Doppler flow probe can easily be placed over the area of tissue that is expected to be infarcted so that a reduction in CBF velocity can be observed with MCAO. With this simple method, it is possible to evaluate the results of MCAO from a reduction of CBF viewpoint. When Belayev et al. (1996) coated the intraluminal suture with poly-L-lysine to make the suture more adherent to the surrounding endothelium, they found that this coating technique resulted in a more consistent infarction. Using the intraluminal filament technique, CBF had been found to decrease by 80% in the cortex and caudoputamen and to remain at this reduced level throughout MCAO, even up to 180 min MCAO (Memezawa et al. 1992). Reperfusion easily occurs when the thread is withdrawn, and the animals may survive for days, weeks, and months, which will enable functional outcome measures to be recorded (DeVries et al. 2001).

The development and proliferation of transgenic and knockout mouse mutants have provided new types of animals to study the genomic and proteomic involvement in mechanisms of cell injury and neuroprotection from ischemia and reperfusion. Similar intraluminal filament techniques developed in rat have been developed in the mouse, and a similar MCAO technique has been developed with the mice being ventilated, anesthetized, and physiologically controlled (Dalkara et al. 1995). Others using this technique have studied transgenic mice and the involvement of a variety of genes and gene products in the mechanism of ische-

mic injury and neuroprotection (Chan et al. 1993; Eliasson et al. 1997; Endres et al. 1997; Huang et al. 1994; Iadecola et al. 2001a,b; Yang et al. 1994). Hattori et al. (2000) have also reported neurobehavior and neurocognitive dysfunction after MCAO in mice.

## Global Cerebral Ischemia Models

In global cerebral ischemia, there is no CBF to any area of the brain, which causes neuronal injury to selectively vulnerable brain areas. Clearly, if global ischemia continued indefinitely, all neurons would die. One of the easiest methods to produce global ischemia without recirculation is decapitation. This technique was used many years ago (Lowry et al. 1964) in small animals to study biochemical mechanisms and pathways in global ischemia; however, it is difficult because it does not lend itself to any modulations. Nevertheless, the decapitated head can be maintained for varying times, and the brain can be freeze-trapped or homogenized for biochemical analysis for metabolic studies (Abe et al. 1983; Ikeda et al. 1986; Yoshida et al. 1985).

The use of a neck tourniquet to produce global ischemia in rats has also been used for many years (Siemkowicz and Gjedde 1980; Siemkowicz and Hansen 1978), but with this technique, there are complicating factors, such as venous congestion and vagal nerve compression, which can lead to variable ischemic outcomes. Neck cuff inflation can also be utilized in large animals (dogs) to produce complete ischemia in the entire brain (Kabat et al. 1941); however, with this technique, the vertebral arteries must be occluded separately because they are encased in vertebrae and will not be occluded by the neck cuff. Using this technique, 2 min of neck cuff inflation resulted in unconsciousness followed by complete recovery. After 4 to 6 min of ischemia, the animals were comatose for 24 hr but did recover normal neurological function (Kabat et al. 1941). After 8 min, permanent neurological deficits were produced (Grenell 1946).

A modification of the neck cuff technique was used in monkeys and included a reduction in arterial blood pressure to 50 mmHg before neck cuff inflation (Nemoto et al. 1977). This technique may be flawed in the sense that the ischemia produced may not be complete because of blood flow via the vertebral and spinal arteries. In this monkey model, injury occurs in brain stem regions (Nemoto et al. 1977), but the pattern and extent of pathology differs from that in other primate models (Miller and Myers 1972; Myers and Yamaguchi 1977). Scheller et al. (1992) used this model in monkeys and found hippocampal damage after 15 min of ischemia, with neurological deficits persisting for many days after the ischemic duration. This neck cuff inflation plus hypotension technique has also been well utilized in cats (Chopp et al. 1987, 1988), in which magnetic resonance spectroscopy could be performed.

Ventricular fibrillation is another technique to produce complete global cerebral ischemia. This technique is used generally in an attempt to mimic the clinical situation of

cardiac arrest, and many investigators have added cardiopulmonary resuscitation (CPR<sup>1</sup>) to follow cardiac arrest (Berkowitz et al. 1991; Dean et al. 1987; Eleff et al. 1991; Koehler et al. 1983; Michael et al. 1984; Safar et al. 1976). Defibrillation is usually successful after ventricular fibrillation, with a combination of chest compressions and administration of epinephrine after an 8- to 12-min cardiac arrest. This technique is generally used in large animals, and although the model is an excellent one, it is expensive and extremely labor intensive because complete intensive care treatment must be provided for the animals, especially during the first 24 to 48 hr after the arrest. In fact, this ventricular fibrillation (cardiac arrest/CPR) model is considered as complete ischemia followed by incomplete ischemia because during the CPR part of the procedure, cerebral perfusion pressure is low and the level of CBF produced is considerably less than the control level (Koehler et al. 1983), despite efforts to increase perfusion pressure via epinephrine. Safar and colleagues have reviewed their extensive experience with outcome (Safar et al. 1990) and neuropathology (Radovsky et al. 1995) in the canine cardiac arrest/CPR model.

Although the dog has been used most frequently for the model described above, the pig has also been utilized often (Berkowitz et al. 1991; Brown et al. 1986, 1987; Schleien et al. 1986). In addition, a new mouse model of cardiac arrest/CPR has recently been presented that utilizes KCl to stop the heart and is complete with CPR procedures (i.e., chest compression, ventilation, and administration of pharmacological agents and physiological monitoring) (Kofler et al. 2002). This model demonstrates clear, consistent injury (with 8-10 min of cardiac arrest) in the hippocampus and the anterior and posterior caudoputamen.

Because ventricular fibrillation results in whole body ischemia, a variety of models have been used that involve occlusion of cephalic arteries of the neck and thorax. These models avoid complete ischemia in the renal, splanchnic, and other peripheral circulations. Hossmann and colleagues (Hossmann 1971; Hossmann and Kleihues 1973; Hossmann and Sato 1970) devised a cat model, which involves occlusion of the innominate and left subclavian arteries near their origin at the aortic arch. The mammary arteries are also ligated, and arterial blood pressure is pharmacologically reduced to less than 80 mmHg, or even 50 mmHg. Cerebral blood flow in this model is reduced to near zero throughout the brain (Clavier et al. 1994; Moskowitz et al. 1989), and as in other ischemic models, pathology and behavior outcome can be titrated to the length of time of the ischemic event. This model has also been extended to monkeys, in which ischemic injury can be produced and partial recovery of cortical function, metabolism, and protein synthesis has been demonstrated (Bodsch et al. 1986; Hossmann and Grosse Ophoff 1986; Hossmann and Zimmerman 1974; Zimmerman and Hossman 1975). These models are somewhat limited in the sense that long-term recovery studies are extremely difficult because of the need for intensive care monitoring and the labor-intensive aspect.

Pulsinelli and Brierley (1979) developed the four-vessel occlusion (4-VO<sup>1</sup>) model to provide a method of reversible forebrain cerebral ischemia in rats. This same group modified the model to increase the percentage of successfully studied rats (Pulsinelli and Buchan 1988; Pulsinelli and Duffy 1983). The often-used 4-VO model can be produced in awake, freely moving rats, but it involves a two-stage procedure to produce the ischemia. In the first stage, on the day before an experiment, atraumatic clamps are placed loosely around each common carotid artery and exteriorized in the neck of the animal. The vertebral arteries are then electrocauterized via the alar foramina of the first cervical vertebra. Unfortunately, it is impossible to visualize these vessels directly, but this occlusion of the vertebral vessels is critical to the success of the model. On the second day, the common carotid arteries are occluded while the animal is awake, and the ischemia is produced. This procedure must result in a complete loss of the righting reflex for the animal to be included in the study. This 4-VO technique is successful in approximately 50 to 75% of the animals, but the effects of ischemia are quite variable between rat strains. It is believed that this variability may be the result of variability of collateral circulation present in each strain (Pulsinelli and Buchan 1988). In the model, CBF changes have been correlated with both the distribution and progression of neuronal damage with ischemia (Pulsinelli et al. 1982).

One hallmark feature of the model described above is that following the reduction in blood flow to less than 3% of control in neocortex, striatum, and hippocampus, a strong cerebral hyperemia is seen 5 to 15 min after reperfusion. Then there occurs a sharp cerebral hypoperfusion, which can last for as long as 24 hr in some brain areas (Pulsinelli et al. 1982). There is also a rather extensive pathology with the 4-VO model. After 30 min of ischemia, striatal neurons are damaged, and hippocampal damage occurs 3 to 6 hr after reperfusion; neocortex damage occurs 1 to 3 days after ischemia (Pulsinelli and Brierley 1979; Pulsinelli et al. 1982). This model has also been utilized for morphological and metabolic studies in anesthetized rats (Dietrich et al. 1984; Globus et al. 1988; Yoshida et al. 1982). The 4-VO model has been utilized by many investigators and has been well validated and well described. It can be utilized in either awake or anesthetized animals, which makes it extremely useful. However, it is not an easy model to use, and there has been much variability in results between laboratories. The success rate of the model is approximately 50 to 75%.

The two-vessel (2-VO<sup>1</sup>) model of forebrain ischemia was developed 25 yr ago (Eklöf and Siesjö 1972a,b) and was initially used to characterize cerebral energy state following incomplete ischemia (Eklöf and Siesjö 1972a; Nordstrom and Siesjö 1987). In this model, bilateral common carotid artery occlusion is coupled with systemic hypotension to produce a reversible forebrain ischemia. Hypotension is produced to achieve an arterial blood pressure of 50 mmHg by bleeding alone (Eklöf and Siesjö 1972a; Kagstrom et al. 1983a; Nordstrom et al. 1978; Smith et al. 1984a), or by

hemorrhage supplemented with pharmacological agents (trimethaphan or phentolamine) (Smith et al. 1984b).

In the 2-VO model, both the ischemia and reperfusion are almost immediate, and the model has been utilized as an alternate to the 4-VO model of forebrain ischemia (Smith et al. 1984b). Cerebral blood flow, when measured between 5 and 15 min of ischemia, is decreased to less than 5% of control in cortex, and to a lesser degree in thalamus and midbrain (Kagstrom et al. 1983b). Following reperfusion, hypoperfusion has been demonstrated (Kagstrom et al. 1983b; Smith et al. 1984b). With this model, injury occurs within selectively vulnerable areas such as the CA1 pyramidal neurons of the hippocampus, caudoputamen, and neocortex (Smith et al. 1984b). After a recovery period of 1 wk, brain damage was observed in hippocampus and subiculum in animals exposed to only 2 min of ischemia, in the neocortex after 4 min, in CA3 after 6 min, and in caudoputamen after 10 min of ischemia (Smith et al. 1984a). In general, this histopathology is similar to that which occurs in the 4-VO model. Like most ischemic models, the variability in histopathology results may be caused by uncontrolled temperature variations, particularly in early investigations, when the importance of temperature control was not completely understood.

It is now well known that production of consistent pathological effects in these models requires very careful control of temperature (Busto et al. 1987). Ginsberg's laboratory has utilized the 2-VO model to study phospholipid and cerebral energy metabolism, to evaluate neurotransmitter metabolism, histopathology, and the effects of temperature manipulations on the histopathology consequences from ischemia (Dietrich 1995; Dietrich et al. 1993; Ginsberg et al. 1992; Globus et al. 1991; Lin et al. 1992; Yoshida et al. 1986). The major advantages of the 2-VO model over the 4-VO model in producing forebrain ischemia are that the 2-VO model requires a more simple surgical preparation, that reperfusion can be readily accomplished, and that this model is easily suitable for chronic survival studies. However, anesthesia and hypotension with drugs are required, which can confound data interpretation.

The production of a global cerebral ischemia mouse model has been difficult. Attempts have been made to apply the rat and gerbil global ischemia models to the mouse, but the success of these attempts appears to be limited. The resulting high level of mortality and frequent complications such as seizures have limited the success of mouse global ischemia. One more recent mouse model of global ischemia involves bilateral common carotid occlusion combined with controlled ventilation (Murakami et al. 1998), and another involves cardiac arrest/CPR (Kofler et al. 2002).

Finally, it is important to mention the gerbil because this animal has been widely used as a model in global ischemia (Kirino 1982). Gerbils have a unique and convenient vascular anatomy, and thus they have been used often in global ischemia studies. The unique anatomical feature of the gerbil is that they do not have a posterior communicating artery, which connects the carotid and vertebrobasilar arterial

system (Levine and Sohn 1969). Thus, global cerebral ischemia in gerbils can be induced by bilateral common carotid artery occlusion. In this bilateral occlusion, CBF is reduced to almost zero in most animals (Crockard et al. 1980; Osbourne and Halsey 1975). In this model, bilateral carotid occlusion of 5 min results in injury and death of the hippocampal CA1 neurons (Kirino 1982).

With unilateral common carotid occlusion, gerbils develop severe neurological signs and die within days (Levine and Payan 1966). Many investigators have demonstrated that approximately 30 to 40% of gerbils develop severe neurological deficits and unilateral infarction after unilateral carotid occlusion (Berry et al. 1975; Harrison et al. 1973; Kahn 1972). In this model, 5 min of ischemia may result in hippocampal injury (Yamamoto et al. 1986). The advantage of these gerbil models is the relative simplicity of the surgical procedure, which allows for the study of many animals. The disadvantages, however, are several. Some animals do not stroke, so the model can be variable; the susceptibility of gerbils to seizures offers a confounding variable into the assessment of ischemic outcome; and, because the animals are small, limited physiological monitoring can be performed.

## Summary

This article provides a review of many (but not all) focal and global cerebral ischemia models currently in use in both small and large animals. Essentially all of these models offer an opportunity to investigate mechanisms, prevention, and treatment (neuroprotection) of ischemic brain injury. With the development of mouse models of ischemia, there is an additional opportunity to examine the effects of genes, gene products, and proteins on outcome from ischemia and to determine which are expressed or not expressed at varying times after ischemia. Whatever model one uses for investigation, however, requires care in measuring and controlling important physiological conditions such as blood pressure, brain temperature, blood gases, and CBF so that infarction size or cell injury is reproducible and consistent. All of these models offer opportunities to study the effects of ischemia on brain. It should also be remembered that when we choose to use animals, we also accept the obligation to care for them properly and treat them respectfully (NRC 1996).

As mentioned above, despite many decades of work with stroke (focal) and global models of cerebral ischemia, only minimal information regarding neuroprotection has been translated to humans. We have learned much from these ischemia models concerning our understanding of complex cellular cascades underlying ischemic injury and possible therapeutic targets. However, it may be that none of the animal models discussed in this manuscript actually closely mimics human ischemia. In addition, the animals used in the models discussed are usually young and healthy, whereas human patients may have other existing patholo-

gies such as age, diabetes, hypertension, and arrhythmia. These other pathologies may alter the way stroke or global ischemia presents in humans, but we have not studied these additional comorbid pathologies in the animal models. Another potential reason for the lack of translation of therapies to humans after ischemia is that the patient clinical trials may not be designed precisely on the basis of preclinical data (e.g., dose of drug, time of administration). An academic/industry joint roundtable has recently made recommendations for appropriate conduct of preclinical trials of neuroprotective agents using animal stroke/ischemic models (stroke therapy) (STAIR 1999).

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