Rodent Models of Cerebral Ischemia

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The use of physiologically regulated, reproducible animal models is crucial to the study of ischemic brain injury—both the mechanisms governing its occurrence and potential therapeutic strategies. Several laboratory rodent species (notably rats and gerbils), which are readily available at relatively low cost, are highly suitable for the investigation of cerebral ischemia and have been widely employed for this purpose. We critically examine and summarize several rodent models of transient global ischemia, resulting in selective neuronal injury within vulnerable brain regions, and focal ischemia, typically giving rise to localized brain infarction. We explore the utility of individual models and emphasize the necessity for meticulous experimental control of those variables that modulate the severity of ischemic brain injury. (Stroke 1989;20:1627-1642)

Why Use Animal Models to Study Cerebral Ischemia?

For the systematic study of the pathophysiology and treatment of cerebral ischemia, it is essential that physiologically controlled, reproducible in vivo animal models be used. There are several reasons for this. 1) Human ischemic stroke is enormously diverse in its manifestations, causes, and anatomic sites; this diversity is resistant to the type of precise analysis and control possible through the use of controlled animal preparations. Furthermore, 2) rigorous histopathologic, biochemical, and physiologic investigations often require invasive surgical procedures and direct access to brain tissue. 3) Events occurring during the first seconds to minutes of an ischemic insult can of necessity be studied only in an animal laboratory. 4) At the heart of ischemia is abnormal perfusion; for its study, presence of the vasculature is essential, a feature lacking in in vitro models such as tissue slices or neuronal/gliai cell cultures.

Although larger animal species (notably cats, dogs, rabbits, and subhuman primates) have been used to study brain ischemia, rodents are equally suitable and, in fact, are more desirable from several vantage points: 1) low cost of the animals, 2) lower cost of procedures that increase in expense as a function of animal weight (e.g., radiotracer or histopathologic techniques), 3) relative homogeneity within strains owing to inbreeding, 4) close resemblance of the cerebrovascular anatomy and physiology to that of higher species, 5) small brain size that is well-suited to such fixation procedures as in vivo freeze-trapping for biochemical analysis, and 6) greater acceptability (compared, for example, with subhuman primates) from both ecologic and ethical perspectives.

We shall consider rodent cerebral ischemia models under a bipartite schema: first, models of global ischemia, usually transient, affecting (by definition) widespread brain areas but typically giving rise to neuronal alterations within selectively vulnerable brain regions; and secondly, models of focal ischemia (with or without reperfusion) eventuating in localized pannecrosis or infarction. The intent of this review is to describe and summarize these models critically, emphasizing those of greatest current utility (Table 1).

Rat Models of Global Cerebral Ischemia

The Two-Vessel Occlusion Model of Forebrain Ischemia

In this model, reversible high-grade forebrain ischemia is produced by bilateral common carotid artery (CCA) occlusions combined with systemic hypotension sufficient to reduce forebrain blood flow markedly. First proposed more than 15 years ago and used to characterize energy state following high-grade incomplete ischemia, this model has received increasing attention in recent years as an alternative to the four-vessel occlusion model (see...
Table 1. Rodent Models of Cerebral Ischemia

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Models of cerebral ischemia in gerbils

| Unilateral CCA occlusion |
| Bilateral CCA occlusions |

CCA, common carotid artery.

below), with which it has been compared.\textsuperscript{10,11} The goal of the two-vessel occlusion model as currently implemented is to produce a "square-wave" insult in which the onset of ischemia and its subsequent reversal are both abrupt.\textsuperscript{10,12} Both CCAs are occluded, and blood pressure is reduced to 50 mm Hg by controlled arterial hemorrhage, for which a servo system may be used.\textsuperscript{13} Trimethaphan or phentolamine may be employed adjunctively to produce hypotension.\textsuperscript{10} Cerebral blood flow (CBF) measured after 5–15 minutes is reduced to <5\% of control in the cerebral cortex, generally to <15\% of control in the caudoputamen, hippocampus, and cingulate cortex, and to a lesser and more variable degree in the thalamus, globus pallidus, and midbrain.\textsuperscript{12} Occasionally, however, considerable side-to-side CBF differences appear even in highly ischemic structures.\textsuperscript{10} During postischemic recirculation, heterogeneous hypoperfusion has been described\textsuperscript{10,12}; regional hemodynamic responses of the postischemic brain to induced hypercapnia and hypoxia have been studied in this model.\textsuperscript{13} The two-vessel occlusion model gives rise to ischemic cell change within selectively vulnerable structures, including the CA1 pyramidal neurons of the hippocampus, caudoputamen, and neocortex.\textsuperscript{11} In general, the histopathology of this model resembles that of the four-vessel occlusion model (see below). Certain histopathologic peculiarities in earlier studies of the two-vessel occlusion model included neuronal injury to the hippocampal CA1 and CA4 pyramidal cells and subiculum with as little as 2 minutes of ischemia; involvement of the neocortex with 4 minutes of ischemia; and injury to the caudoputamen with 8–10 minutes of ischemia, together with side-to-side differences in the extent of hippocampal pathology with short periods of ischemia.\textsuperscript{11} As previous workers controlled rectal temperature but were unaware of the sensitivity of ischemic neurons to variations in brain temperature,\textsuperscript{14} they may have induced these abnormalities by inadvertent exposure of the cranium to asymmetric warming.\textsuperscript{10,11}

We have used the two-vessel occlusion model to study phospholipid and energy metabolism in ischemia\textsuperscript{15} and, more recently, to evaluate neurotransmitter metabolism, histopathology, and the protective effects of mild cerebral hypothermia.\textsuperscript{16} As bilateral CCA occlusions alone (in normotensive strains) are insufficient to reduce CBF into the ischemic range\textsuperscript{17} or to perturb the energy state of the tissue,\textsuperscript{6} the success of this model is critically dependent upon the production of adequate hypotension. From our personal experience with this model, we suspect that the precision with which blood pressure reduction is regulated during ischemia is crucial to achieving a consistent outcome and that rather minor fluctuations around the accepted level of 50 mm Hg may produce variable neuropathology.

To summarize, the two-vessel occlusion model has the advantages of one-stage surgical preparation; production of high-grade forebrain ischemia; ability to control ventilation so as to ensure normoxia and normocarbia; ease of instituting cerebral recirculation; suitability for chronic survival studies; and probably a lower experimental failure rate (from either acute death or an inadequate extent of ischemia) than appears to be associated with the four-vessel occlusion model (see below). The disadvantages of the two-vessel occlusion model include the need for anesthesia and induced hypotension; the use of anesthetics and drugs may complicate the interpretation of outcome. There is some interanimal inconsistency with respect to both CBF decrement as well as pathologic outcome. Postischemic seizures may occur occasionally following longer periods of ischemia.\textsuperscript{10} Finally, this model cannot be used in awake animals, so behavioral alterations immediately after occlusion cannot be assessed.

The Four-Vessel Occlusion Model of Forebrain Ischemia

This widely employed model allows high-grade forebrain ischemia to be produced in awake, freely moving Wistar rats, and the resulting neuropathology tends to be reproducible. This model is produced in two stages.\textsuperscript{18} In the first stage, under anesthesia an "atraumatic arterial clasp"\textsuperscript{19} is placed around each CCA and exteriorized through a ven-
entral midline neck incision. Through a dorsal incision, the alar foramina of the first cervical vertebrae are identified; beneath these foramina pass the two vertebral arteries. A small monopolar electrocautery needle is then inserted through each foramen to electrocoagulate the vertebral arteries. In a report published 9 years after the initial description of this model, additional important technical detail is provided, namely, that the rat’s head should be placed in stereotactic bar and tilted downward by approximately 30° to the horizontal, and that the cervical spine should be extended by gentle tension on the rat’s tail to bring the plane of the alar foramina to the horizontal for improved visualization. Successful electrocoagulation of the vertebral arteries is essential to the eventual success of this model, yet it is impossible to view these vessels directly. It has been our experience that considerable technical skill and finesse are required during the first-stage procedure; for example, it is possible for a less than fully skilled operator to injure the brainstem while coagulating the vertebral arteries.

Forebrain ischemia is produced in the second-stage procedure 24 hours later. The awake rat may be hand-held or momentarily restrained while the carotid clamps are tightened; these clamps may later be removed to permit reperfusion. Among rats with previous vertebral artery occlusion, the addition of bilateral CCA occlusions gives rise to prompt unresponsiveness and loss of the righting reflex in approximately three fourths of cases; only these rats are considered to have been rendered adequately ischemic. An isoelectric electroencephalogram (EEG) should appear in these unresponsive rats within 2–3 minutes of bilateral CCA occlusion and persist until recirculation. Lack of critical ischemia in the brainstem is suggested by the persistence of spontaneous respirations and preservation of the corneal reflex. Postischemic seizures are not observed with 10 minutes of ischemia, but following 30 minutes of ischemia seizures occur in approximately 20% of rats at 24 hours, and in 40% at 72 hours; convulsing rats are generally excluded from further analysis.

It should be noted that unsuccessful outcomes occur in approximately 25% of the rats; in two thirds of these failures the rat fails to become fully unresponsive, and in the remainder the rat dies from respiratory failure within 2–3 minutes. Importantly, Pulcinelli and Brierley found it necessary to test Wistar rats from several suppliers to find a strain yielding a high incidence of successful ischemia. Differences in collateral blood supply were postulated to be responsible for this observed variability, although other sources of variation (e.g., dietary) are also possible.

Other laboratories have reported even greater variability. Blomqvist et al carried out the four-vessel occlusion model in two different rat strains. In standard Wistar rats, animals surviving the first-stage procedure suffered respiratory arrest within 3 minutes after bilateral CCA occlusion in two thirds and one half of the experiments, respectively, when the interval between the first and second stages was 1 and 2–4 days. While Sprague-Dawley rats suffered no respiratory arrest, almost one half of the animals in that strain developed postischemic status epilepticus and only 50–60% exhibited coma, signifying high-grade ischemia.

Pulcinelli and Buchan acknowledge that rats from the same strain but different suppliers, or even different shipments of rats from the same supplier, may vary in their response to four-vessel occlusion, presumably on the basis of variable collateral blood supply and areas of critical hypoperfusion in the brainstem. This group has suggested that a suture placed just anterior to the cervical and paravertebral muscles be tightened partially during bilateral CCA occlusion so as to interrupt the cervical collateral blood flow; however, overtightening of this suture may lead to death from respiratory arrest and brainstem ischemia.

This model has also been used successfully in anesthetized rats for morphologic and metabolic investigations. When so used, the first-stage procedure need include only bilateral vertebral artery occlusion. One day later, the carotid arteries can be both isolated and transiently occluded in a single procedure. The superimposition of mild hypotension (approximately 80–90 mm Hg) offsets the transient initial hypertension induced by CCA occlusions and helps to make the outcome more uniform. Although anesthesia precludes the observation of clinical signs as predictors of successful ischemia, a persistently isoelectric EEG is strongly suggestive.

Histopathology has been extensively assessed in this model. With 10 and 20 minutes of ischemia, vulnerable zones of the hippocampus show ischemic cell change in approximately 40% and 85% of the hemispheres, respectively, after 3 days of survival. Consistent neuronal injury to the striatum requires at least 20–30 minutes of ischemia, and these changes are maximally expressed by 24 hours. In common with other global ischemia models, the four-vessel occlusion model is less effective in producing neocortical histopathology, with a broad spectrum of severity resulting from even 30 minutes of ischemia.

Radiotracer studies during ischemia have shown local CBF (ICBF) to be markedly reduced in the striatum and neocortex (<3% of control) and in the hippocampus (3–7% of control). Diencephalic and cerebellar CBFs are rather less severely affected (10–15% of control), and ICBF in the brainstem is maintained at approximately 25–30% of control; the latter is probably obligatory if acute death from brainstem ischemia is to be averted. In laboratories experiencing difficulty with this model, however, substantially higher ICBF values in the forebrain have been reported, together with side-to-side CBF differences and intrastructural variability. One group has suggested that moderate hypotension...
sion (50 mm Hg) be superimposed to produce consistently severe ischemia. One cannot exclude the possibility, however, that the latter workers did not adequately coagulate the vertebral arteries during the first-stage procedure.

As is characteristic of reperfusion following global ischemia, early hyperemia is followed by post-ischemic hypoperfusion, lasting approximately 1 hour in the hippocampus and striatum but lasting up to 6 hours in the parietal neocortex. Local cerebral glucose utilization (lCMRgl) 1 hour after ischemia is generally depressed in brain regions that were moderately to severely ischemic. The fractional recovery of early postischemic lCMRgl has been reported to exceed that of early ICBF in the hippocampus, striatum, and dorsolateral cortex. The possibility that the disproportionate recovery of lCMRgl relative to ICBF during the early post-ischemic period predisposes to selective vulnerability is supported by findings in another model of hemispherical ischemia.

Behavioral studies in the four-vessel occlusion model have shown a partial impairment of "working" memory and a transient alteration of "reference" memory following 30 minutes of ischemia—deficits probably reflecting hippocampal injury. A variant of the four-vessel occlusion model, entailing electric current-induced clot formation in one CCA, has been used to demonstrate the ameliorative effects of indomethacin and prostacyclin.

To summarize, the four-vessel occlusion model has been solidly validated and is widely employed to produce reversible high-grade incomplete forebrain ischemia. The advantages of this model include applicability to either awake or anesthetized rats. A major disadvantage, however, is the inability of all laboratories to achieve satisfactory results with this model, even when the presumably optimally suited Wistar strain is employed. (As will be reviewed below, unsuspected inconsistency of brain temperature during and following ischemia may explain at least some of this variability among laboratories.) Even in the best hands, only approximately 50% of the rats successfully survive the first-stage procedure, have forebrain ischemia of a sufficiently high grade, and avoid the possible complications of acute death from brainstem ischemia and postischemic seizures.

Ischemia Models Involving Elevated Cerebrospinal Fluid Pressure

Bihemispheral forebrain compression-ischemia models. Earlier studies of cerebral ischemia in larger animals expended much effort to define the duration of complete circulatory interruption to which the brain was tolerant—an area of inquiry giving rise to highly discrepant estimates. Ljunggren and colleagues extended this work to rats by adapting an earlier procedure for inducing complete brain ischemia by infusing artificial cerebrospinal fluid (CSF) into the cisterna magna so as to elevate CSF pressure above arterial blood pressure by 20-70 mm Hg; reflex hypertension was prevented by infusing the ganglionic blocking agent trimethaphan. These maneuvers predictably deplete forebrain glucose stores by 1 minute and markedly reduce adenosine triphosphate (ATP) concentration and elevate tissue lactate content by 3-5 minutes.

With the reversal of ischemia, phosphocreatine and ATP occurs within 90 minutes, but this is less than complete if the ischemic period has exceeded 5 minutes. This model has been used to study CBF, glycolytic and Krebs cycle metabolites, and tissue acidosis.

Yoshida et al adapted the concept of compression-ischemia to compare the sequelae of complete with those of incomplete cerebral ischemia when both conditions were produced in a comparable manner. This was accomplished by bilateral CCA occlusions, halothane-induced hypotension (mean arterial blood pressure 50-60 mm Hg), and elevation of CSF pressure; the latter was raised to 90-130 mm Hg (i.e., above systolic blood pressure) to produce complete ischemia and to 10-15 mm Hg to produce incomplete ischemia. Profound tissue lactic acidosis was avoided in both conditions, and it was shown that the persistence of residual CBF during ischemia aided recovery of energy metabolism in subcortical regions during recirculation. Correspondingly, neuropathologic sequelae were more severe in rats with complete than in those with incomplete ischemia. The incomplete-ischemia version of this model has been used to study monoamine neurotransmitters in ischemia.

Graded unihemispheral ischemia. Busto and Ginsberg combined temporary unilateral CCA occlusion with elevation of CSF pressure (to 40-45 mm Hg by intracisternal infusion of mock CSF) and maintenance of mean arterial blood pressure at 100-110 mm Hg by controlled hemorrhage as required. These procedures reduce regional CBF by 85-90% in the dorsolateral and lateral neocortex, the hippocampus, and the lateral caudoputamen ipsilateral to the occlusion and induce a graded pattern of energy metabolite change, with marked metabolite depletion in the lateral cortex, hippocampus, lateral striatum and thalamus; and intermediate energy metabolite levels in the dorsolateral cortex, the medial striatum, and the medial thalamus. Lactate concentration is elevated in all neocortical areas. Thus, this model appears to give rise to a consistent "metabolic penumbra" (the dorsolateral cortex), characterized by marked lactic acidosis but only intermediate energy metabolite depletion. Cerebral perfusion in the contralateral hemisphere is sufficient to avert metabolic and histologic abnormalities.

Double-isotope radiotracer studies performed during the early postischemic period reveal parallel depressions of both regional CBF and CMRgl in the previously ischemic neocortex, whereas in the stri-
atium there is early resumption of normal CMRgl in the face of hypoperfusion, resulting in marked metabolism/blood flow uncoupling.32 Correspondingly, the striatum is the predominant locus of ischemic cell change in this model, raising the possibility of a causal connection with the posts ischemic uncoupled state.32

To summarize, this model has the advantages of graded, unihemispheric ischemia in a consistent topographic distribution, avoidance of microsurgery and craniotomy, reversibility of the insult, and the absence of seizures. However, a demanding multistep surgical procedure is necessary to produce ischemia. In addition, mock CSF infusion leads to CSF rhinorrhea, probably due to rupture of the cribriform plate, a complication that would eventually predispose to meningitis and thus preclude chronic survival studies. A closely related model of "hemodynamic" cerebral ischemia combining unilateral CCA occlusion with hemorrhagic hypotension (but without elevated CSF pressure) has been described, however, which also gives rise to ipsilateral neuronal pathology.51

Miscellaneous Ischemia-Producing Strategies

Global ischemia by neck tourniquet. By inflating a pressure cuff around the neck of an anesthetized rat to very high pressures (approximately 600–700 mm Hg), CBF may be reduced to <1% of control throughout the brain.52 Arterial blood pressure is regulated at 60 mm Hg during ischemia by blood withdrawal/infusion. As expected, this model occludes both cranial arteries and veins and subjects other cervical structures to great pressure. It has been used occasionally53 but has not found widespread acceptance.

Decapitation ischemia. Decapitation instantaneously induces a condition of irreversible global brain ischemia. The decapitated head may be maintained at 37°C for desired time intervals, and the brain may then be freeze-trapped or homogenized for biochemical analysis. We have used this model extensively in metabolic studies.64,54–56

The Levine Preparation of Hypoxia-Ischemia and Its Modifications

Levine’s introduction of "anoxic-ischemic encephalopathy" was a solution to the problem of how to expose rats to hypoxia of a degree sufficient to damage the brain while avoiding the systemic complications of hypotension, cardiac arrhythmias, and death.57 The original model consisted of unilateral CCA ligation in Wistar rats, followed 1 day later by gradual exposure to an anoxic environment for up to 45 minutes, without physiologic monitoring. The insult gave rise to variable lesions of both the gray and the white matter, with the hippocampus proving most vulnerable.57

This model was subsequently refined by instituting careful physiologic monitoring and control, and cerebral metabolites and histopathology were characterized.58,59 Unilateral CCA ligation in the absence of hypoxemia leaves the cerebral energy state unchanged, but unilateral CCA occlusion plus hypoxemia (Pao2 21 or 28 mm Hg) gives rise to tissue lactic acidosis and a decline in the concentrations of high-energy phosphates, more severe on the side of the occlusion; these changes tend to reverse after termination of the insult, but to a less complete degree in the ipsilateral hemisphere of the more severely hypoxic rats. The pattern of high tissue concentrations of glycolytic intermediates together with reduced concentrations of certain Krebs cycle constituents suggests that the effect of unilateral CCA occlusion in this model is merely to exaggerate the influence of hypoxia rather than to interfere with the substrate supply, as would be expected in a purely ischemic insult.58 This surmise is supported by studies revealing, in general, increases in CBF during this insult, of greater magnitude on the side without occlusion.58,60,61 That is, this model produces a condition of relative oligemia (i.e., CBF insufficient for proper oxygen provision) in the CCA-clamped hemisphere. Neuropathologic abnormalities in the physiologically controlled Levine preparation are found only on the side of the occlusion and consist of selective ischemic neuronal change affecting the MCA-supplied cortex, hippocampus, and striatum—areas generally corresponding to sites of relatively low CBF.64 These data suggest that the pattern of selective damage in this model may be determined by differences in the regional severity of the relative oligemia. It has been further shown that the neuronal injury in this model occurs without evidence of focal vascular nonperfusion.62

The Levine model has also been used to produce hypoxia-ischemia in immature rats,63 but without the ability to monitor physiologic variables in these small animals the influence of unobserved systemic factors in producing injury cannot be excluded. Furthermore, small animal size impedes the quantitative application of radiotracer techniques.64 The Levine model in immature rats has been used to study neurotransmitters,55 tissue oxidation-reduction state,66 and protein synthesis.64

To summarize, the Levine model is rather straightforward to produce, although maintenance of normotension during the hypoxic period may be difficult, and secondary hypotension may confound the interpretation of outcome. This model is well suited to side-to-side comparison of the effects of hypoxia with the effects of the superimposed relative oligemia. The caveat in using this model, however, is that the brain is not truly ischemic, and hence, findings in this model cannot necessarily be generalized to the setting of true global brain ischemia.

Rat Models of Focal Cerebral Ischemia

Middle Cerebral Artery Occlusion

Rat models of MCA occlusion have gained increasing acceptance in recent years owing to their rele-
vance to the human clinical setting. A number of studies published during the past decade have served to characterize this model in great detail and to provide important insights into sources of variability (Table 2). The subtemporal approach of Tamura et al68 and its modifications70,73,79 have emerged as a standard method of proximal MCA occlusion, giving rise to infarction of both the cortex and the caudoputamen. Bederson et al70 contributed crucial observations as to how the precise site and extent of MCA occlusion influence neurologic and neuropathologic outcome. Their findings argue persuasively that the lenticulostriate arteries and the small cortical branches must be isolated from both proximal and distal sources of collateral blood supply for consistent infarcts to result (Table 2). The superimposition of moderate arterial hypotension has the effect of consistently enlarging the resultant infarct.73

The consequences, in Sprague-Dawley rats, of MCA occlusion by electrocoagulation proximal to the origins of the lateral lenticulostriate arteries have been well characterized in several recent reports.99,81,82 In general, a very close topographic correspondence has been observed between the zone of reduced regional CBF and the area of histologic abnormalities.81 Interestingly, a striking 10-fold transition of ICBF is evident over a 1–2-mm interval at the edge of the pathologic lesion, supporting the notion that the ischemic "penumbra" is probably quite narrow.82 The mean CBF in areas of consistent damage in these studies is 25 ml/100 g/min.81,82

Models of proximal MCA occlusion have been applied broadly to assess the effects of pharmacotherapeutic agents such as calcium channel blockers69,72 and excitatory amino acid antagonists.80 Temporary MCA occlusion has been achieved by means of a reversible snare ligature,79 although this technically demanding procedure has not found wide application. An alternative approach to reversible MCA occlusion makes use of a variant of the photochemical method (see below), in which thrombosis is induced in an MCA segment by laser illumination following systemic administration of rose bengal.74,83 The occlusive thrombus is composed of aggregated platelets and erythrocytes.76,84 Prompt recanalization is achieved following the topical application of nimodipine to the occluded segment; when instituted after 1 hour, the neocortex is largely preserved from infarction, although a mixed pattern of infarction and ischemic cell change is evident in the striatum.76

In a different rat strain (Long-Evans), Chen et al71 employed a surgically less demanding, more distal occlusion of the MCA above the rhinal fissure,67,85 coupled with permanent ipsilateral and temporary contralateral CCA occlusions. The latter were found to be necessary to reduce CBF in the MCA territory into the ischemic range (mean value, 18% of control).71 Protracted survival is possible with this model; neuropathologically, brains show moderate-sized infarcts in the frontoparietal cortex, sparing the caudoputamen (Table 2).71

Brint et al77 have used a "rapid tandem" method, consisting of MCA occlusion distal to the rhinal fissure coupled with permanent occlusion of the ipsilateral CCA, to compare quantitative histopathology in three rat strains. Cortical infarct volumes tended to be variable and inconsistent in both Wistar and Fischer-344 rats, but the infarcts were larger and more reproducible in spontaneously hypertensive rats (SHR) (Table 2).

In a recent comprehensive analysis of MCA occlusion, Duverger and MacKenzie78 employed the modified method of Tamura et al68,79 to assess the sequelae of permanent MCA occlusion in several strains (Table 2). These authors observed no seizures. Pathologically, all strains showed infarcts in the caudoputamen. The cortical region most consistently infarcted was the olfactory cortex, with more variable involvement of the frontoparietal somatosensory cortex. Among the normotensive strains, mean infarct volume was smallest and most variable in Wistar-Kyoto rats, somewhat larger and more consistent in Sprague-Dawley rats, and largest and most consistent in Fischer-344 rats. The SHR and stroke-prone spontaneously hypertensive rat (SHRSP) strains showed 1.5–1.6 times the hemispheric infarct volume of the Fischer-344 strain and low (7–8%) coefficients of variation (Table 2).78 Hence, the Fischer-344 strain was regarded as the normotensive strain of choice. In that strain, hyperglycemia increased infarct size by approximately 1.4 times, whereas infarct size was largely unaffected by age. Studies of regional energy metabolism in Fischer-344 rats revealed three distinct regions: 1) the striatum, the site of severe, early, and nonremitting changes; 2) the parietal cortex, in which energy metabolites showed an early moderate derangement that worsened at 48 hours and persisted somewhat at 7–14 days; and 3) cortical regions lying outside the infarct, in which energy state was largely preserved.86

Nedergaard87 assessed the extent to which selective neuronal injury is present in cortical regions adjacent to the zone of pan necrosis. (In human stroke cases, within a few days after onset a zone of selective acidophilic neuronal alterations has been described at distances up to approximately 5–10 mm from the infarct border.)80 In rat models of MCA occlusion, this region pertains to a zone <1 mm from the infarct.89 Paradoxically, hyperglycemia appears to lessen neuronal injury in peri-infarct zones whereas hypoglycemia is detrimental in this respect, possibly owing to the influence of brain glucose on the modulation of spreading cortical depression.90,91

To summarize, rat models of MCA occlusion are highly useful approximations of ischemic hemispheric infarction in humans and, as such, will undoubtedly receive continued attention, particularly in efforts to substantiate the therapeutic efficacy of drugs prior to initiating human clinical trials. Despite a confusing plethora of variants of this model (Table 2), certain general points can be
Pathologic proliferation. Microaneurysms have been noted in thickened basement membranes, and fibroblastic vascular smooth muscle with fibrinoid degeneration, features include injury to the endothelium and vasculature of these strains differs morphologically and physiologically from that of normotensive rats (see below). As such, it is conceivable that drugs that diminish focal infarction in normotensive rats might fail to do so in SHR. Thus, negative therapeutic trials in the latter strain should not dissuade an investigator from repeating his observations in normotensive rats. 3) Complete physiologic monitoring, including attention to brain temperature and plasma glucose level (see below) as well as to arterial blood pressure and blood gases, is required to ensure an interpretable outcome in this and other models of ischemia. Several of these issues have been addressed critically in a recent editorial.92

The Spontaneously Hypertensive Rat as a Stroke Model

Although SHR are not normally prone to spontaneous stroke,93 they are unusually susceptible to various cerebrovascular occlusive procedures that might be tolerated in normotensive strains. For example, SHR exhibit much more profound CBF declines following bilateral CCA occlusions94 and frequently develop large cerebral infarcts.95 Even in young SHR, marked CBF reductions and energy metabolite depletion result from 3 hours of bilateral CCA occlusions.96 As reviewed above, MCA occlusion gives rise to much larger cortical infarcts in SHR than in normotensive strains.77,78,97 This susceptibility precedes the establishment of fixed hypertension and thus appears not to be secondary to it.97

Okamoto et al93 first established a stroke-prone strain of SHR (SHRSP), in which both cerebral hemorrhages and infarcts occur spontaneously in >80% of the males. Control of blood pressure reduces this stroke incidence.98 Major sites with predilection for stroke in SHRSP include the anteromedial and occipital cortex and the basal ganglia.99 The common angioarchitectural features at these sites appear to be 1) recurrent branchings, that is, blood vessels in which the spatial vector of blood flow is opposite to that of the parent vessel; and 2) the involvement of vascular boundary zone areas. These features suggest obvious parallels with human stroke.

Microangiopathic characteristics of SHR and SHRSP include multifocal blood–brain barrier (BBB) opening, with leakage of plasma proteins into the brain parenchyma at blood pressures exceeding approximately 210–220 mm Hg: the predominant sites are the cerebral cortex and the basal ganglia, most commonly in border zone areas.100 Pathologic features include injury to the endothelium and vascular smooth muscle with fibrinoid degeneration, thickened basement membranes, and fibroblastic proliferation. Microaneurysms have been noted in zones of BBB injury, whereas regions without marked BBB leakage show more minor changes.100 The pathogenesis of these vascular changes is thought to be directly related to chronic elevation of intraluminal pressure. Sites of BBB leakage are associated with spongy-edematous pathologic alterations of the brain parenchyma, necrotic cysts, ingress of phagocytic cells, astrocytosis, and some vacuolated or acidophilic neurons. Extensive signs of focal ischemic injury, however, are generally absent.101

Painstaking physiologic studies have revealed SHRSP to have elevated vascular resistance in larger cerebral arteries, which tends to attenuate increases in microcirculatory pressure during chronic hypertension.102 Structural factors account in part for this increased resistance. Chronic sympathectomy removes the structural component of resistance in the larger cerebral vessels and reduces the vasoconstrictor response to acute blood pressure increases.102,103 Stroke incidence in SHRSP is reduced in the denervated hemisphere following unilateral superior cervical ganglionectomy at 1 month but not at 3 months of age.103

CBF studies have shown that following MCA occlusion, normotensive (Wistar-Kyoto) rats maintain effective vasodilator reserve in the collateral vessels, whereas SHRSP regularly develop large infarcts in the MCA territory, despite the fact that these two strains have similar numbers of anastomotic vessels joining the MCA and anterior cerebral artery beds.104 With maturation, hypertrophy of pial arterioles occurs more markedly in SHRSP and is associated with a progressive rightward shift of the stress–strain relation of pial arterioles with respect to normotensive rats.105 In addition, impaired endothelium-mediated dilatation may impair collateral vasodilator responses during chronic hypertension.106

Genetic crossing studies have shown that the enhanced susceptibility of SHRSP to cerebral infarction following MCA occlusion is accounted for by a single gene locus, with infarct size in SHRSP progeny being proportional to the “dose” of SHRSP present.107 Compared with normotensive rats, even young SHRSP have grossly larger infarcts following MCA occlusion, before developing severe hypertension.108 This susceptibility to infarction persists in SHRSP despite antihypertensive medications, whereas normotensive Wistar rats made acutely hypertensive are still largely protected from infarction following MCA occlusion.109 These data suggest that the susceptibility of SHRSP to infarction may not be explained entirely by the vascular lesions of chronic hypertension.

Dietary factors strongly influence the development of strokes in SHRSP.96,110 A low-protein diet of fish origin, coupled with 1% saline drinking water, is associated with average systolic blood pressures exceeding 250 mm Hg and with stroke in all (male) animals by 3 months of age.110 Interestingly, Tobian et al111 have shown a dramatic 98% reduction in mortality and complete prevention of
### Table 2. Rat Models of MCA Occlusion: Representative Morphologic Studies

<table>
<thead>
<tr>
<th>Authors (year)</th>
<th>Rat strain</th>
<th>Vessel(s) occluded and anatomic sites</th>
<th>Duration</th>
<th>Regions infarcted</th>
<th>Infarct size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Robinson and Coyle (1980)</td>
<td>Sprague-Dawley</td>
<td>MCA-O distal to rhinal fissure, via frontoparietal craniotomy</td>
<td>Perm.</td>
<td>C: frontoparietal cortex above rhinal fissure</td>
<td>Diameter ~1-5 mm</td>
</tr>
<tr>
<td>Tamura et al (1981)</td>
<td>Sprague-Dawley</td>
<td>Subtemporal craniectomy with zygoma removal. MCA-O between rhinal branch and lateral striate arteries</td>
<td>Perm.</td>
<td>C: lateral caudate, frontal cortex I: sensorimotor and auditory cortex (75%), medial caudate (38%)</td>
<td>Not measured</td>
</tr>
<tr>
<td>Mohamed et al (1985)</td>
<td>Sprague-Dawley</td>
<td>MCA-O via Tamura method but with zygoma intact</td>
<td>Perm.</td>
<td>C: lateral cortex and striatum, similar to Tamura et al</td>
<td>Hemispheric volume $47\pm7\text{ mm}^3$ (mean±SEM)</td>
</tr>
<tr>
<td>Bederson et al (1986)</td>
<td>Sprague-Dawley</td>
<td>MCA-O: long segment from origin to inferior cerebral vein</td>
<td>Perm.</td>
<td>C: cortex, basal ganglia</td>
<td>Extensive</td>
</tr>
<tr>
<td>Bederson et al (1986)</td>
<td>Sprague-Dawley</td>
<td>MCA-O: from 2 mm proximal to olfactory tract to inferior cerebral vein</td>
<td>Perm.</td>
<td>C: cortex, basal ganglia</td>
<td>Extensive</td>
</tr>
<tr>
<td>Chen et al (1986)</td>
<td>Sprague-Dawley</td>
<td>Focal MCA-O at origin from ICA</td>
<td>Perm.</td>
<td>I: (13%)</td>
<td>—</td>
</tr>
<tr>
<td>Chen et al (1986)</td>
<td>Sprague-Dawley</td>
<td>Focal MCA-O 1 mm distal to inferior cerebral vein</td>
<td>Perm.</td>
<td>None</td>
<td>—</td>
</tr>
<tr>
<td>Gotoh et al (1986)</td>
<td>Sprague-Dawley</td>
<td>MCA-O (ligation) superior to rhinal fissure, plus permanent ipsilateral CCA-O and 1-hr contralateral CCA-O</td>
<td>Perm.</td>
<td>C: large MCA-territory infarcts in dorsolateral and lateral frontoparietal cortex (96%); occasional small contralateral MCA infarcts</td>
<td>Average area of surface infarct $100\pm6\text{ mm}^2$ (mean±SEM), maximal cross-sectional area $10.4\pm1.1\text{ mm}^2$ (mean±SEM)</td>
</tr>
<tr>
<td>Gotoh et al (1986)</td>
<td>Long-Evans</td>
<td>MCA-O as above with permanent ipsilateral CCA-O only</td>
<td>Perm.</td>
<td>I: small variable cortical infarcts</td>
<td>Maximal cross-sectional area $4.8\pm1.9\text{ mm}^2$ (mean±SEM)</td>
</tr>
<tr>
<td>Gotoh et al (1986)</td>
<td>Long-Evans</td>
<td>MCA-O alone</td>
<td>Perm.</td>
<td>No grossly visible infarct</td>
<td>Maximal cross-sectional area $1.7\pm0.8\text{ mm}^2$ (mean±SEM)</td>
</tr>
<tr>
<td>Osborne et al (1987)</td>
<td>Sprague-Dawley</td>
<td>MCA-O just medial to olfactory tract, by modified Tamura method</td>
<td>Perm.</td>
<td>C: dorsolateral cortex, lateral striatum</td>
<td>Cortex: $29\pm10\text{ mm}^3$, striatum: $22\pm2\text{ mm}^3$ (mean±SEM)</td>
</tr>
<tr>
<td>Osborne et al (1987)</td>
<td>Sprague-Dawley</td>
<td>Exposure as per Tamura but without zygoma removal. MCA-O (coagulation and division) just medial to olfactory tract</td>
<td>1) Normotensive throughout</td>
<td>C: lateral and inferolateral cortex, lateral striatum</td>
<td>$11-25%$ of hemisphere (range)</td>
</tr>
<tr>
<td>Osborne et al (1987)</td>
<td>Sprague-Dawley</td>
<td>2) Blood pressure lowered to 60 mm Hg (by hemorrhage) for 30 min, beginning 15-20 min after MCA-O</td>
<td>Perm.</td>
<td>C: inferolateral, lateral, and dorsolateral cortex; lateral and central striatum</td>
<td>$24-35%$ of hemisphere (range)</td>
</tr>
<tr>
<td>Prado et al (1988)</td>
<td>Wistar</td>
<td>Photochemical MCA-O (rose bengal, argon ion laser) just distal to rhinal branch, plus ipsilateral CCA-O for 2 hr and contralateral CCA-O for 1 hr</td>
<td>Perm.</td>
<td>C: dorsolateral and lateral frontoparietal cortex I: dorsolateral striatal crescent</td>
<td>Cortex: $32-82\text{ mm}^3$ (range)</td>
</tr>
<tr>
<td>Prado et al (1988)</td>
<td>Sprague-Dawley</td>
<td>Photochemical MCA-O (rose bengal, argon ion laser) just proximal to rhinal branch; no CCA-O</td>
<td>Perm.</td>
<td>C: dorsolateral and central striatum I: overlying cortex (highly variable)</td>
<td>Striatum: $14-89\text{ mm}^3$ (range)</td>
</tr>
</tbody>
</table>
TABLE 2. (Continued)

<table>
<thead>
<tr>
<th>Authors (year)</th>
<th>Rat strain</th>
<th>Vessel(s) occluded and anatomic sites</th>
<th>Duration</th>
<th>Regions infarcted</th>
<th>Infarct size</th>
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</thead>
<tbody>
<tr>
<td>Nakayama et al</td>
<td>Sprague-Dawley</td>
<td>Modified subtemporal approach&lt;sup&gt;46&lt;/sup&gt;; MCA-O 2 mm long, proximal to olfactory tract, containing lenticulostriate origins</td>
<td>Perm.</td>
<td>C: dorsolateral and lateral frontoparietal cortex, dorsolateral and central striatum</td>
<td>Cortex: 85±22 mm&lt;sup&gt;3&lt;/sup&gt;, striatum: 30±6 mm&lt;sup&gt;3&lt;/sup&gt; (mean±SD)</td>
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<tr>
<td>Nakayama et al</td>
<td>Sprague-Dawley</td>
<td>Photochemical MCA-O (rose bengal, He-Ne laser); thrombosis extending from olfactory tract to just distal to rhinal branch</td>
<td>Perm.</td>
<td>C: dorsolateral and lateral frontoparietal cortex, central and dorsolateral striatum</td>
<td>Cortex: 33±10 mm&lt;sup&gt;3&lt;/sup&gt;, striatum: 20±2 mm&lt;sup&gt;3&lt;/sup&gt; (mean±SEM)</td>
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<tr>
<td>Duverger and MacKenzie</td>
<td>Sprague-Dawley</td>
<td>Same, but recanalization induced by topical nimodipine following 1-hr MCA-O</td>
<td>Temp.</td>
<td>C: dorsolateral striatum</td>
<td>Cortex: 3±2 mm&lt;sup&gt;3&lt;/sup&gt;, striatum: 11±3 mm&lt;sup&gt;3&lt;/sup&gt; (mean±SEM)</td>
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<td></td>
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<td>I: central striatum; minimum cortical pathology</td>
<td></td>
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<td></td>
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<td></td>
<td>Range of mean cortical infarct volumes:</td>
<td></td>
</tr>
<tr>
<td>Brint et al</td>
<td>Wistar</td>
<td>MCA-O (cautery) just superior to rhinal fissure, plus permanent ipsilateral CCA-O</td>
<td>Perm.</td>
<td>I: small variable cortical infarcts</td>
<td>48–209 mm&lt;sup&gt;3&lt;/sup&gt; (avg. CV=68%)</td>
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<tr>
<td></td>
<td>Fischer-344</td>
<td>Same</td>
<td>Perm.</td>
<td>I: larger, less variable cortical infarcts</td>
<td>38–110 mm&lt;sup&gt;3&lt;/sup&gt; (avg. CV=99%)</td>
</tr>
<tr>
<td></td>
<td>SHR</td>
<td>Same</td>
<td>Perm.</td>
<td>C (&gt;50%): moderate or large cortical infarcts</td>
<td>157–259 mm&lt;sup&gt;3&lt;/sup&gt; (avg. CV=19%)</td>
</tr>
<tr>
<td></td>
<td>SHR</td>
<td>MCA-O without CCA-O</td>
<td>Perm.</td>
<td>I: small lesions, dorsolateral caudoputamen (67%)</td>
<td>243±14 mm&lt;sup&gt;3&lt;/sup&gt; (mean±SEM)</td>
</tr>
<tr>
<td>Duverger and MacKenzie</td>
<td>Wistar</td>
<td>Proximal MCA-O (coagulation and division) via subtemporal craniectomy; zygoma intact&lt;sup&gt;68,79&lt;/sup&gt;</td>
<td>Perm.</td>
<td>C: caudoputamen in all groups</td>
<td>162±18 mm&lt;sup&gt;3&lt;/sup&gt; (mean±SEM)</td>
</tr>
<tr>
<td>Graham et al</td>
<td>Sprague-Dawley</td>
<td></td>
<td>Perm.</td>
<td>C: inferolateral cortex</td>
<td>Hemisphere infarct volume:</td>
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<td></td>
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<td></td>
<td></td>
<td>I: lateral cortex</td>
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<tr>
<td></td>
<td>Wistar-Kyoto</td>
<td>Same</td>
<td>Perm.</td>
<td>C: inferolateral and lateral cortex</td>
<td>62 mm&lt;sup&gt;3&lt;/sup&gt; (CV 49%)</td>
</tr>
<tr>
<td></td>
<td>Sprague-Dawley</td>
<td>Same</td>
<td>Perm.</td>
<td>I: dorsolateral cortex</td>
<td>99 mm&lt;sup&gt;3&lt;/sup&gt; (CV 33%)</td>
</tr>
<tr>
<td></td>
<td>Fischer-344</td>
<td>Same</td>
<td>Perm.</td>
<td>C: inferolateral and lateral cortex</td>
<td>106 mm&lt;sup&gt;3&lt;/sup&gt; (CV 20%)</td>
</tr>
<tr>
<td></td>
<td>SHR</td>
<td>Same</td>
<td>Perm.</td>
<td>C: extensive cortical infarct (inferolateral, lateral, dorsolateral)</td>
<td>158 mm&lt;sup&gt;3&lt;/sup&gt; (CV 8%)</td>
</tr>
<tr>
<td></td>
<td>SHRSP</td>
<td>Same</td>
<td>Perm.</td>
<td>C: extensive cortical infarct</td>
<td>172 mm&lt;sup&gt;3&lt;/sup&gt; (CV 7%)</td>
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<tr>
<td></td>
<td>Sprague-Dawley</td>
<td>Proximal MCA-O via subtemporal craniectomy&lt;sup&gt;68,70&lt;/sup&gt;</td>
<td>Perm.</td>
<td>C: dorsolateral cortex, striatum</td>
<td>Cortex: 92±6 mm&lt;sup&gt;3&lt;/sup&gt;, striatum: 24±1 mm&lt;sup&gt;3&lt;/sup&gt; (mean±SEM)</td>
</tr>
</tbody>
</table>

MCA-O, middle cerebral artery occlusion; ICA, internal carotid artery; CCA-O, common carotid artery occlusion; Perm., permanent; Temp., temporary; C, constant (100%); I, inconsistent; SHR, spontaneously hypertensive rats; SHRSP, stroke-prone SHR; CV, coefficient of variation.

cerebral hemorrhage in SHRSP fed a potassium-supplemented diet. Although this diet lowered blood pressure by an average of approximately 45 mm Hg, the data suggest that this may not be the sole basis of the protective effect.<sup>111</sup>

To summarize, the predisposition of SHR and SHRSP to develop large cortical infarcts following MCA occlusion makes these strains useful in controlled experimental studies, although the investigator should be cognizant that the cerebral vasculature of these strains differs from that of normotensive strains. By contrast, the spontaneous strokes occurring in SHRSP are more difficult to study by virtue of their greater variability of timing, location, and pathologic features. Nonetheless, SHRSP may be useful in morphologic investigations of the structural-vascular antecedents of ischemic infarction and intracerebral hemorrhage.

**Photochemically Induced Focal Cerebral Thrombosis**

Following the administration of a photosensitizing dye, platelet aggregation can be induced in an organ by irradiating it with light of a specific wavelength.<sup>112</sup> Watson et al adapted this approach so as to engender thrombotic infarction of the rat...
cerebral cortex. The dye rose bengal is injected intravenously, and the scalp of an anesthetized rat is retracted. Light at 560 nm delivered to the skull penetrates into the brain, where it interacts photochemically with the intravascular rose bengal to generate singlet oxygen, which peroxidizes susceptible lipid molecules within the vascular endothelium and blood elements and gives rise to microvascular platelet aggregation; direct thermal injury is avoided.

A unique advantage of this model is that the infarct may be placed in any desired cortical location. Lesion size and depth depend upon the intensity of the irradiating beam, the duration of irradiation, and the dose of rose bengal administered. The typical infarct is a sharply circumscribed, bowl-shaped necrotic lesion occupying the full cortical thickness but sparing underlying structures. Morphologically, within minutes of light exposure cortical regions destined for infarction show extensive platelet aggregation within pial and intraparenchymal vessels, swollen endothelial cells containing altered organelles, and focally damaged plasma membranes. Early BBB breakdown and vasogenic edema give rise to progressive microvascular compression at the lesion periphery. Microvascular perfusion failure is evident within minutes, and the lesion doubles in size during the first 4 hours.

The reproducibility of lesion size and location lends this model to radiotracer studies of the local and remote hemodynamic and metabolic sequelae of infarction and their pharmacologic modification. The precision of lesion localization in this model has allowed it to be used to examine postinfarction functional responsiveness of specific neural systems and pharmacologic modification of functional recovery in stroke. In other applications, this model has been used to study calcium channel blocker therapy in stroke and has yielded unexpected evidence of a slight protective effect of hyperglycemia in the setting of microvascular occlusion. A photochemical technique has also been used to generate a useful model of graded spinal cord infarction.

To summarize, the photochemical model of thrombotic infarction has several advantages: 1) a straightforward, minimally invasive procedure permitting chronic animal survival, 2) a consistent lesion size, 3) the ability to place an infarct in any chosen cortical location, and 4) the involvement of endothelial injury and platelet aggregation, conveying the potential for this model to be used in studies of antplatelet or thrombolytic therapy. Disadvantages of the model include 1) its end-arterial occlusive nature, which makes the lesion resistant to therapies based on the enhancement of collateral perfusion, and 2) prominent microvascular injury, early BBB opening, and vasogenic edema—features atypical of large-vessel occlusion models and, arguably, not representative of human thrombotic stroke.

**Miscellaneous Models of Cerebral Embolism and Thrombosis**

**Blood clot embolization.** Injection of blood clots into the carotid artery, used for decades to produce embolic stroke in larger species, has recently been adapted to rats. Injection of homologous blood clot fragments <100 μm in size into the CCA of rats leads to a high incidence of ipsilateral infarcts involving the cerebral cortex, hippocampus, and deep gray structures. By placing a retrograde cannula into the external carotid artery, with its tip at the entrance to the internal carotid artery (ICA), blood clots may be introduced into the ICA without obstructing its flow. In a clever adaptation of this method, human blood clot was used to produce MCA-territory embolism in rats to test the thrombolytic efficacy of human recombinant tissue plasminogen activator.

**Microsphere embolization.** The injection of 35-μm carbon microsphere emboli into the ICA of rats has been used to document early ipsilateral depression of cerebral energy metabolism and the evolution of cerebral edema in this multifocal infarction model.

**Photochemically initiated thromboembolism.** The photochemical method has recently been modified so as to induce nonocclusive platelet thrombosis in the CCA of rats; the carotid lesion gives rise to distal embolization, leading to focal cerebral infarction. Lesion width ranges from 0.1 to 1.7 mm; infarcts are found predominantly in the cerebral cortex and less often in the hippocampus, thalamus, and basal ganglia.

**General comments regarding embolic stroke models.** Although embolic stroke models in rats are useful for specific purposes (e.g., the study of clot thrombolysis or the cerebral pathology of platelet microembolization), the random and unpredictable location and size of lesions in embolic models tend to restrict their utility for the study of stroke pathomechanisms.

**Arachidonate-induced thrombosis.** The injection of sodium arachidonate into the ICA of rats induces unilateral intravascular cerebral thrombosis. Histopathology has not been adequately documented in this model, and its general utility has not yet been established.

**Gerbil Models of Cerebral Ischemia**

**Unilateral Common Carotid Artery Occlusion**

Levine and Payan first observed that approximately 20% of Mongolian gerbils (Meriones unguiculatus) subjected to unilateral CCA ligation developed severe neurologic signs and died within 2 days. Subsequent histologic studies revealed an absence of the expected posterior communicating arteries that, in other species, connect the carotid and vertebrabasilar arterial systems. Numerous studies have established the propensity of gerbils to develop severe neurologic deficits and unilateral
hemispheric infarction in approximately 30–40% of the animals following ipsilateral CCA ligation and have confirmed the absence of “significant” posterior communicating arteries.134–136 Smaller (30–60 μm) vessels, however, coursing on the ventral surface of the midbrain, provide communications between the basilar and carotid circulations and are sufficient to permit some passage of intravascular dye into the carotid system despite bilateral CCA occlusions.137

In an important study of the anatomic basis of clinical variability in this model, Berry et al.138 established that, whereas all gerbils show a lack of anastomoses between the anterior and posterior circulations, those animals capable of surviving unilateral CCA ligation with only minor sequelae have, in addition, a prominent anastomosis between the proximal portions of the two anterior cerebral arteries, typically forming a midline azygous vessel. In gerbils becoming severely symptomatic, the latter communications are uncommon.139

In clinically affected gerbils, hemispheric swelling, pallor, and softening evolve over the first 4–24 hours following CCA occlusion; in comatose gerbils, extensive hemispheric infarction is observed by 24 hours.138 However, ischemia for as little as 5 minutes may give rise to dendritic abnormalities in the hippocampus.139

Varying degrees of unresponsiveness, hemiparesis, circling, and clonic convulsions as well as generalized “rolling fits” are observed clinically.135 Seizures are prominent in this model and are consistent with the genetic susceptibility of gerbils to convulse even in the absence of ischemia.140,141

The chief advantage of the unilateral CCA occlusion model in gerbils is the obvious convenience of the simple surgical procedure, permitting many animals to be studied—a seductive advantage, for example, in screening cerebroprotective agents. Against this, however, must be weighed numerous disadvantages: 1) Gerbils are so small (50–80 g) that physiologic monitoring procedures routinely possible in rats become technically daunting (e.g., arterial and venous catheterization, endotracheal intubation) or else are physiologically destabilizing (e.g., repeated blood sampling, as is required in autoradiographic tracer studies). Hence, gerbil experiments often tend to be physiologically unmonitored and hence are of restricted value in interpreting ischemic pathomechanisms. 2) Since stroke occurs only one third of the time, experimental protocols necessitate that gerbils be awakened following CCA occlusion to judge their clinical state. 3) The susceptibility of this species to seizures introduces a confounding variable into the assessment of ischemic outcome.

**Bilateral Common Carotid Artery Occlusions**

The lack of posterior communicating arteries in gerbils133–135 also renders this species susceptible to high-grade bilateral forebrain ischemia following bilateral CCA occlusions. Forebrain CBF falls to near zero in most animals,142 with others having residual CBF of approximately 10 ml/100 g/min.143 Importantly, it was shown in this model that transient CCA occlusion lasting as little as 5 minutes results in delayed cell death of the hippocampal CA1 pyramidal neurons,144 a finding also demonstrated in rats,28,145 which provided the initial in vivo support for the excitotoxic theory of ischemic cell death.146 Longer periods of ischemia tend to accelerate the evolution of ischemic cell change.147 Another pathologic process, selective chromatolysis, has been described in gerbils, predominating in the deeper cortical layers and in the CA1 area and the paramedian zone of the hippocampus.148 This change, identical to the “reactive change” of Ito et al.,149 appears to be related, not to the duration of ischemia per se, but rather to epileptic seizures triggered by the ischemic insult.148

Principally owing to its convenience, the bilateral CCA occlusion model in gerbils has been used extensively in studies of stroke pathomechanisms150–155 and pharmacoprotection154,155 and in characterization of repetitive ischemic insults.156 The several disadvantages of gerbils discussed above, however, pertain as well to the bilateral CCA occlusion model of ischemia.

**Factors Modulating the Severity of Ischemic Injury**

**Relevance of Plasma Glucose Level**

Myers and Yamaguchi157 first noted serendipitously that rhesus monkeys administered a glucose load before 15 minutes of cardiac arrest developed profound neurologic abnormalities, brain edema, and widespread cerebral gray matter necrosis, whereas comparable food-deprived monkeys exhibited only minimal signs and restricted neuropathologic abnormalities. Subsequent reports have abundantly confirmed that hyperglycemia at the time of an ischemic insult markedly exacerbates brain injury in models of both global145,158–161 and focal74,90,162,163 ischemia. The anaerobic metabolism of increased glucose stores during ischemia appears to mediate this deleterious effect via the production of marked tissue lactic acidosis, which tends to be more severe when the degree of ischemia is incomplete, owing to continued glucose delivery to the ischemic brain.45,159,161 Concomitants of this process include marked accentuation of postischemic hypoperfusion and depletion of brain energy metabolites.45,158,159,161 Even moderate elevations of blood glucose concentration are sufficient to worsen outcome.160 From insightful microelectrode studies, it has been inferred that hydrogen ion buffering appears to be compartmentalized within glial cells during ischemia164,165 and that the transition from isolated ischemic neuronal pathology to frank tissue necrosis may be mediated by the exhaustion of glial buffering capacity and the subsequent rupture of cell membranes.166

By analyzing different models of focal ischemia,74,123 we have shown that hyperglycemia...
increases infarct volume significantly in tissue zones receiving collateral circulation but has no deleterious influence in regions of end-arterial perfusion. Paradoxically, at the periphery of an infarcted focus, hyperglycemia may protect penumbral neurons from ischemic change. 10

To summarize, hyperglycemia is an important modulatory factor in models of brain ischemia, so that care must be taken to monitor and report plasma glucose levels whenever ischemia models are investigated; the failure to have done so may account for at least some of the variability observed in previous reports. The use of fasted animals tends to minimize (though not eliminate) interanimal variability of plasma glucose levels.

The Importance of Brain Temperature in Animal Ischemia Models

Recent studies from our laboratory have clearly established that 1) brain temperature may vary independently of core body temperature during a cerebral ischemic insult, 2) small variations in brain temperature during and after ischemia markedly influence the extent of ischemic neuronal pathology, and consequently, 3) care must be taken to monitor and control brain temperature when such models are studied. 14 Our studies were prompted by observations of a disconcerting degree of interanimal variability in our own and others' 11, 16 studies. In global ischemia, we have shown that moderate-to-marked injury to the CA1 hippocampal pyramidal neurons occurs in 100% of brains held at 36°C during 20 minutes of ischemia but that the percentage injury falls to 20% of brains at 34°C and to 0% at 33° and 30°C. In a similar fashion, the extent of ischemic injury to the dorsolateral striatum is reduced by approximately 80% at 33–34°C and is completely eliminated at 30°C. 14 This protective effect cannot be explained by differential alterations of CBF or concentrations of energy metabolites or free fatty acids during ischemia, 14, 16 but it may be related to our observation that mild hypothermia almost totally suppresses intras ischemic release of the excitatory neurotransmitter glutamate and partially blunts the release of dopamine. 26 Importantly, in recent studies we have shown that moderate hypothermia instituted immediately after ischemia also confers marked histologic protection. 16

These observations carry the unequivocal message that failure to monitor and control brain temperature, or allowing body temperature to vary during ischemia or following the administration of therapeutic agents, 16 would be expected to introduce unacceptable variability into the outcome of experimental ischemia studies. As brain temperature in rats, particularly during and after ischemia, cannot be predicted from a knowledge of rectal temperature alone, it must be independently monitored. 14 Disturbingly, most previous experimental studies of brain ischemia in animal models have failed to monitor and control brain temperature; as such, their conclusions must be viewed circumspectly.

Concluding Comments

Rodent models of both global and focal cerebral ischemia offer a fertile milieu for investigating the mechanisms, prevention, and treatment of ischemic brain injury. With increased awareness that the failure to control and regulate physiologic variables modulating the severity of ischemia may yield erroneous or misleading data, it is now incumbent upon investigators using animal ischemia models to be critically attuned to the necessity that physiologic monitoring and control of these models be meticulously carried out; that the application of biochemical, physiologic, or behavioral methods to these models be rigorous, quantitative, and statistically valid; and that the ethical and compassionate treatment of animals be observed.

Acknowledgment

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M D Ginsberg and R Busto

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