by the Robinson group. Some rats have very large Evans blue leaks that are most likely a result of MCA occlusion. For most old rats, the thicker skull requires more drilling, and connective tissue about the vessel presents more resistance to dissection of the MCA making hemorrhage more likely than in younger rats. While the smaller lesions in older rats (734-852) are larger \((p < 0.05)\) than for the younger rats (752-842), the size difference may be due to more surgical trauma in exposing the vessel or some age factor. Since neither the Robinson nor the Tamura group differentiated the size of the lesion due to surgery from the one due to the occlusion, a good comparison of data is not yet possible.

Very large infarcts (mean size > 60 mm\(^2\)) invariably occur after MCA occlusion above the rhinal fissure in young spontaneously hypertensive stroke-prone rats (SHRSP) but not normotensive controls and are reported in a forthcoming paper. Factors that may be involved are discussed more fully and include rat strain, age, blood pressure, location of the occlusion, altered cerebral metabolism, reduced blood flow due to inadequate or insufficient regulation of collaterals, and so forth. Therefore, a single factor or a multifactorial combination involving method of occlusion, metabolic, vascular structural or hemodynamic alterations with age may be responsible for infarcts after rapid MCA occlusion.

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References

Cerebral Blood Flow in the Four-Vessel Occlusion Rat Model

To the Editor:

In a recent article appearing in this Journal, Furlow\(^1\) reported measurements of regional cerebral blood flow (CBF) during four-vessel occlusion (4-vessel occlusion) in the rat. Since his results are substantially different from previously-published values\(^2\) and since his experimental methods violate our original description of the model,\(^4\) we feel obliged to restate the nature and degree of blood flow changes attained when the correct experimental conditions are followed.

Four-vessel occlusion in the rat\(^4\) has proven to be a highly reproducible method to achieve reversible but near-total forebrain ischemia.\(^2\) As a result, the model has yielded a consistent pattern of morphological brain damage following transient ischemia,\(^5\) that correlates well with regional changes in glucose metabolism\(^2\) and high-energy metabolite levels.\(^6\) This method for producing forebrain ischemia in the rat has been adopted by a number of investigators, and in some cases it has been modified to meet the needs of the individual experiments. Unfortunately, in certain instances, such modifications have resulted in a failure to follow the criteria which identify animals meeting the definition of successful 4-vessel occlusion.

Briefly described, the method involves production of forebrain ischemia by permanent occlusion of the vertebral arteries in the anesthetized animal and then 24 hours later temporary occlusion of the common carotid arteries in the awake animal. Since the animals are awake it is possible to observe the behavioral response of every animal subjected to this procedure. Only animals that become unresponsive and completely lose their righting reflex for the duration of the carotid artery occlusion are accepted as meeting the definition of 4-vessel occlusion.\(^4\) Immediately after and just prior to terminating occlusion of the carotid arteries the animals are placed on their left and right sides and stimulated with a tail or hindpaw flick to assure complete, bilateral loss of the righting response. Animals that fail to meet this test are excluded from the study. Approximately 75% of Wistar rats (Hilltop Farms) show complete loss of the righting response upon 4-vessel occlusion.\(^4\) In those instances where the animals are to be paralyzed and mechanically ventilated during 4-vessel occlusion, the rapid appearance of an isoelectric EEG\(^4\) will, in most cases, assure severe forebrain ischemia. Dilatation of the pupils upon occlusion of the carotid arteries has also proven to be a useful criterion of successful 4-vessel occlusion in either awake or paralyzed animals. Thus every animal subjected to 4-vessel occlusion, whether awake or paralyzed-ventilated at the moment of carotid artery occlusion, must meet these specific criteria or be excluded from further study.

When the above criteria are strictly followed, cerebral blood flow is reduced to the level previously reported by us\(^2\) and reproduced in Table 1. When measured with \(^14\)C-iodoantipyrine, blood flow to most of the forebrain during 4-vessel occlusion is reduced to approximately 3% or less of control values, while blood flow to the diencephalon, cerebellum and brainstem ranges from 10% to 30% of control values. Ginsberg,\(^3\) using the same CBF tracer and similar criteria for selection of ischemic animals, reported virtually identical degrees of regional CBF reduction in awake rats during 4-vessel occlusion.

Furlow\(^1\) reported measurements of regional CBF in paralyzed-ventilated rats using both polarographic and radioactive tracer techniques. Normal values obtained with the hydrogen clearance method varied widely between the two nonnormotensive control groups, and the absolute CBF values obtained with this method were substantially lower than his control values obtained with the \(^14\)C-iodoantipyrine method. For these reasons and because the hydrogen clearance method is relatively inaccurate in severely ischemic tissue, we will restrict our comments to the CBF values obtained with the \(^14\)C-iodoantipyrine method. Furlow reports that CBF to various forebrain regions was reduced to only 40–70% of control values during 4-vessel occlusion. We can state categorically that such animals will not show an isoelectric EEG and therefore do not conform to the definition of 4-vessel occlusion as originally described. Animals that maintain a partial righting response during 4-vessel occlusion and that appear behaviorally to be only lethargic or stuporous will

<table>
<thead>
<tr>
<th>Region</th>
<th>Control (6)</th>
<th>4-Vessel occlusion (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal neocortex</td>
<td>133 ± 8</td>
<td>1 ± 0.1</td>
</tr>
<tr>
<td>Parietal neocortex</td>
<td>140 ± 9</td>
<td>1 ± 0.2</td>
</tr>
<tr>
<td>Striatum</td>
<td>97 ± 3</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>80 ± 4</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>Diencephalon</td>
<td>111 ± 10</td>
<td>11 ± 3</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>107 ± 12</td>
<td>17 ± 4</td>
</tr>
<tr>
<td>Brainstem</td>
<td>112 ± 14</td>
<td>30 ± 2</td>
</tr>
</tbody>
</table>

Values (derived from reference 2) represent mean ± SEM for the number of animals shown in parentheses.

Table 1 Regional Cerebral Blood Flow (ml/100 gm/min) in Control Rats and Rats During 4-Vessel Occlusion

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continue to show slow wave activity on the EEG (unpublished observations). Measurements of CBF in animals with the latter behavioral and EEG characteristics revealed that forebrain CBF was reduced to only 40–60% of control values (Pulsinelli et al., unpublished observations; Ginsberg2). Thus Furlow’s failure to monitor each and every animal and to exclude those not meeting the above criteria resulted in the measurement of CBF in animals that fail to satisfy the conditions of successful 4-vessel occlusion.

In the original description of the 4-vessel occlusion rat model4 we indicated that the source of residual CBF during occlusion of the carotid and vertebral arteries must arise from collateral circulation through the cervical and paravertebral muscles. In animals subjected to successful 4-vessel occlusion, as defined above, collateral blood flow through the anterior spinal artery maintains cerebellar and brainstem blood flow at 15 and 30% of normal values respectively, but contributes little flow to the forebrain structures.2 In animals that fail to become unresponsive upon 4-vessel occlusion, continued blood flow to the forebrain must arise either from vessels lying in the cervical or paravertebral muscles or from incomplete occlusion of the carotid or vertebral arteries. Failure to occlude the carotid or vertebral arteries is not a problem in our hands since occlusion of both pairs of vessels is accomplished under direct visual observation. In the case of the vertebral arteries, the canals in the first cervical vertebra, through which the arteries travel, are observed through the dissecting microscope to be empty after electrocauterization. Furlow1 indicates that postmortem analysis of two random animals prior to his CBF study revealed that one vertebral artery was patent in the first animal and both vertebral arteries were patent in the second animal. He attributes this to his method of electrocauterization of these vessels which does not allow visualization of the cautery process, and from incomplete occlusion of the carotid or vertebral arteries. Failure undoubtedly succumb to severe brainstem ischemia.2

Insofar as preparatory technique is concerned, direct visualization through the foramen transversarium via the alas foramen to ensure obliteration of each vertebral artery would be a tour de force in most Sprague- Dawley rats. The alas foramina are usually minute and not properly aligned with the optical axis of the operating microscope over the small surgical field. Consequently, I used a tamponade of bone wax to minimize the likelihood of patent vertebral arteries after electrocoagulation. With regard to the method of carotid occlusion, the original model of Pulsinelli and Brierley2 called for reopening the day-old ventromedial cervical incision in the awake animal.4 Herein lies a potentially serious problem in the model, for Pulsinelli and Brierley took great pains to describe the same phenomenon.2 Both investigators even had difficulty maintaining their “success” rate in rats from the same anonymous supplier, and they attributed this curious result, without evidence, to the possibility that “inbreeding may have improved the collateral supply to the brain.”2 Such unpredictability among animals may, of course, affect the reliability and acceptance of the model.

My study1 was prompted by the absence in the original report by Pulsinelli and Brierley2 of a quantitative determination of cerebral blood flow, an oversight made up in print some 2–3 years later.3, 4 Perfusion of the arterial system with an unspecified blue dye gave an estimate of vascular patency, but hardly of the magnitude of tissue perfusion. Thus, one learns that, despite the previous assertion that the brainstem is spared during bilateral vertebrocarotid occlusion,2 arteriographic measurements show definite, albeit variable, declines in blood flow through the brainstem. Indeed, the “useful criterion” of pupillary dilation that follows carotid occlusion can be explained by ocular and/or brainstem ischemia, whereas the 8% of rats dying of acute respiratory failure undoubtedly succumb to severe brainstem ischemia.2

In their critique of my work, Pulsinelli and his colleagues rebuff my results and reiterate their own. Yet, they have missed the obvious explanation for the glaring disparity between my results and theirs: I used Sprague-Dawley rats instead of the Wistar rats requisite for the model strictu sensu. This omission by the Cornell group is indeed surprising in that I propose this explanation in my paper, and Pulsinelli and Brierley took great pains to describe the same phenomenon.2 Herein lies a potentially serious problem in the model, for, Pulsinelli and Brierley had to test “Wistar rats from several suppliers . . . before finding a strain that resulted in a similar 70–80% success rate observed earlier.”2 These two investigators even had difficulty maintaining their “success” rate in rats from the same anonymous supplier, and they attributed this curious result, without evidence, to the possibility that “inbreeding may have improved the collateral supply to the brain.”2

Editor’s Note: The previous letter was submitted to the author for comment.

To the Editor:

It is unfortunate that investigators must define a “successful four-vessel occlusion” differently in New York and Birmingham. Living closer to the equator, perhaps I took a more simplistic view of semantics: to my thinking, a “successful four-vessel occlusion” is achieved when one obstructs both common carotid and both vertebral vessels so that blood no longer flows directly through them to reach the brain. However, as Pulsinelli, Levy, and Duffy intimate, bilateral vertebrocarotid occlusion is not tantamount to high-grade cerebral ischemia. I agree. In fact, this conclusion is a major point of my paper.1 My study examined the effect of the Pulsinelli-Brierley mode of four-vessel occlusion on brain blood flow.2 The authors of the above letter (two of whom were seemingly omitted from the original report?) emphasize that the Pulsinelli-Brierley model of “successful four-vessel occlusion” is something else again because it consists of a surgical technique and a post-surgical selection procedure.2 The essence of the technique is the innovative, intra-Atlantic electrocoagulation of the vertebral arteries when combined subsequently (24 hr ad litteram) with conventional, reversible occlusion of the common carotid arteries. Once the animals have undergone four-vessel occlusion, the Cornell group insists upon application of various selection criteria. These criteria introduce the intentional bias of eliminating animals whose cerebral ischemia is insufficient for the ad hoc purposes of the investigators. Based on behavioral findings during ischemia, Pulsinelli and his collaborators discard at least every fourth animal to ensure homogeneity of the animal preparation. When animals are followed for 72 hr after ischemia, they further exclude, because of convulsions, up to 40% of rats subjected to 30 min of successful four-vessel occlusion. In other words, fewer than half of the 30-min animals are retained by the end of study. Clearly, the ultimate price of “success” can be costly when failures outnumber successes because of various restrictions.

References


occlude the ipsilateral carotid is ascertained at the time of placement of the ligatures. This method permits reversible arterial occlusion in a highly reliable fashion that does not require visual confirmation.

In conclusion, I feel my analysis of brain blood flow is a fair characterization of the effects of bilateral vertebrocarotid occlusion in the Sprague-Dawley rat without the selection biases for "success" imposed by Pulsinelli and co-workers. I do not wish to downplay the novelty and potential utility of the Pulsinelli-Brierley model. It is certainly one of the better models for producing cerebral ischemia in the rat, but like every model it possesses idiosyncrasies and limitations. I trust this discussion aids in clarifying some of the details of the method so necessary for overcoming such shortcomings.

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References
Cerebral blood flow in the four-vessel occlusion rat model.
W A Pulsinelli, D E Levy and T E Duffy

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