
Experimental Air Embolism of the Brain: An Analysis of the Technique in the Rat

THOMAS W. FURLOW, JR., M.D.

SUMMARY Air embolization of the brain produces cerebral ischemia that can be focal and reversible. The method has previously been hampered by (1) lack of selective arterial injection of the embolus, (2) disruption of local hemodynamic relationships by ligation of major arterial channels, (3) excessive volume of the air embolus, and (4) uncontrolled bubble size. To minimize these factors, a technique was devised in the rat whereby a fine catheter was advanced through a branch of the external carotid artery into the ipsilateral cerebral hemisphere for seconds to a couple of minutes. The duration of ischemia varied from region to region, and it tended to be prolonged by arterial hypotension. In the nonembolized hemisphere, CBF never declined abruptly (indicating no cross-over of air) although electrical activity was suppressed in two-thirds of the animals.

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INJECTION OF AIR into the arterial vasculature of the brain is a convenient technique for producing focal and potentially reversible ischemia in animals. Many investigators have used the method through the years but under widely varying experimental conditions (table 1). Perusal of these reports reveals one or more methodological limitations in many of the animal models. First, the site of the arterial injection has at times lacked selectivity. As a result, the embolus of air distributes itself randomly into two or more major arteries, including those supplying noncerebral tissues, and the full volume of air may thus fail to reach the brain. Second, hemodynamic relationships between adjacent arterial territories have been needlessly disturbed during catheterization or puncture of the artery to be injected. For example, a common practice has been to ligate the external carotid artery3,4,19 or the common carotid artery2,13,16,18 before or after injection of air. Ligations of this type lead to alterations of flow through anastomotic channels that may cause a drop in the perfusion pressure in the tissue supplied by the ligated artery. It is, therefore, not surprising that blood is diverted from the internal carotid artery into the
territory supplied by the external carotid artery after ligation of the latter. Third, the total volume of the injected air has occasionally exceeded the intravascular capacity of the embolized arterial tree. Hence, 'spill-over' of air, for example, into the contralateral hemisphere, or, even worse, into the vertebrobasilar territory, not only diminishes the focal nature of the model, but also may render the animal preparation unstable because of brainstem ischemia or widespread embolization. Fourth, the size of individual air bubbles emerging from the intra-arterial catheter or needle has usually not been controlled. Large bubbles, of course, possess neither the surface area nor the surface activity of an equivalent volume of small emboli that may be encountered clinically. One approach to reducing the size of air bubbles is to use blood foam for embolization. Rationally, however, any technique for air embolization should take into account the four parameters that influence the production of uniform microbubbles in vitro: (1) the size of the orifice from which the air emerges, (2) the pressure of injection, (3) the velocity of liquid flow past the orifice, and (4) the surface tension of the flowing liquid. Of these parameters, only the first two can be controlled in vivo.

The model of air embolism described in this paper has overcome many of the limitations imposed by other methods. By preserving reasonably normal hemodynamic balance between adjacent arterial supplies of the head and brain, one is able to produce focal, high-grade ischemia with microliter volumes of air.

**Materials and Methods**

Under halothane-oxygen anesthesia 50 male, Sprague-Dawley rats (Charles River Farms) weighing 300–450 g underwent a two-stage procedure consisting of implantation of intracerebral electrodes and catheterization of the internal carotid artery.

**Electrode Implantation and Hydrogen Polarography**

Epoxy-coated (epoxy resin, A-M Systems, Inc., Everett, WA 98204), platinum-10% iridium electrodes (5 mil diameter, Engelhard, Carteret, NJ 07008) with an exposed 1-mm tip were stereotactically implanted into the rostral thalamus, the caudal hippocampus, and the lateral neocortex of both cerebral hemispheres. The pin connectors (Amphenol 220-S03, Bunker Ramo, Danbury, CT 06810) of the electrodes were embedded in methylmethacrylate cement to form a damage-resistant cap. During experiments, electrodes were polarized at +650 mV. After saturation of the brain to a breathing mixture containing 7% hydrogen, the hydrogen was turned off and the descent of the hydrogen-induced current was recorded. Regional CBF in ml/100 g/min was calculated as the quotient of 69.3 divided by the time in minutes for the clearance curve of hydrogen to decline halfway from its maximum potential during tissue saturation to zero indicating complete washout of the hydrogen indicator. Further details are available elsewhere.

**Catheterization of the Internal Carotid Artery**

A fine-gauge catheter was fabricated by drawing out polyethylene tubing (PE-10, Clay Adams, Parsippany, NJ 07054) heated over a soldering iron. A bead for tying the catheter in place was formed by heating the junction of the tubing and its attenuated tip (O.D. 0.20 ± 0.02 mm, I.D. 80 ± 5 microns). The catheter was manipulated by external version into the internal carotid artery. Return of arterial blood was verified before ligating the catheter above and below the bead (fig. 1A). Under an operating microscope the first branch of the right external carotid, viz., the posterior occipital artery, was dissected free, doubly ligated with 6x0 silk suture and divided between the ligatures leaving a 3-mm arterial stump. The stump angled toward the operator and under slight traction, the catheter tip was passed through a small nick in the wall of the arterial segment by means of a right-angled catheter introducer fashioned from no. 33-gauge stainless-steel hypodermic tubing (Small Parts, Inc., Miami, FL 33138). The catheter was advanced with fine forceps toward the heart just beyond the carotid bifurcation. The catheter tip was gently manipulated by external version into the internal carotid artery. Return of arterial blood was verified before ligating the catheter above and below the bead (fig. 1B). The other end of the catheter was exteriorized by tunneling it subcutaneously to emerge through the skin of the nape of the neck. The catheter was used immediately, or it was filled with heparin and heat-sealed for later use.

**Table 1**

<table>
<thead>
<tr>
<th>Species</th>
<th>Route of injection*</th>
<th>Volume of air (ml)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ape</td>
<td>CCA; CCA &amp; L/ECA</td>
<td>0.1–4</td>
<td>1-4</td>
</tr>
<tr>
<td>Cat</td>
<td>CCA; CCA &amp; L/CCA; CCA &amp; L/ECA; IA</td>
<td>0.1–4</td>
<td>1, 2, 5–9</td>
</tr>
<tr>
<td>Dog</td>
<td>CCA; CCA &amp; L/ECA; VA; ICA &amp; L/ICA; IV</td>
<td>0.05–21</td>
<td>10–15</td>
</tr>
<tr>
<td>Gerbil</td>
<td>ICA &amp; L/CCA</td>
<td>0.03–0.05</td>
<td>16, 17</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>CCA</td>
<td>0.1–0.2</td>
<td>5</td>
</tr>
<tr>
<td>Rabbit</td>
<td>CCA; CCA &amp; L/CCA; IV</td>
<td>0.01–4.0</td>
<td>5, 12, 18</td>
</tr>
<tr>
<td>Rat</td>
<td>CCA &amp; L/ECA &amp; L/PPA</td>
<td>0.005</td>
<td>19</td>
</tr>
</tbody>
</table>

*Abbreviations: CCA = common carotid artery, ECA = external carotid artery, IA = innominate artery, ICA = internal carotid artery, IV = intravenous, PPA = pterygopalatine artery. An ampersand denotes 'combined with,' and * L denotes ligation of a vessel before or after injection of air.

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I—10 mm—90 mm

FIGURE 1. (A) A diagram showing the dimensions of the specially made polyethylene catheter used in this study. (B) Catheterization of the internal carotid artery (ICA). The catheter has been passed into the posterior occipital artery (POA), a branch of the external carotid artery (ECA). The pterygopalatine artery (PPA) has been ligated, and the common carotid artery (CCA) is fully patent.

Animal Monitoring

After insertion of a tracheostomy tube and catheters (PE-50, Clay Adams, Parsippany, NJ 07054) into the femoral arteries and veins, animals were withdrawn from halothane anesthesia, paralyzed with D-tubocurarine (5 U subcut), and connected to a rodent ventilator (model 680, Harvard Apparatus, Millis, MA 02054). Oxygen supplementation was given to maintain the arterial oxygen tension above 90 Torr. The arterial tension of carbon dioxide was held to 35 ± 5 Torr whenever possible, and the arterial pH was maintained between 7.35 and 7.40. Blood gases were serially measured from 100-μl samples of arterial blood (Micro 13, Instrumentation Laboratory, Lexington, MA 02173). Body temperature was maintained at 37.5° ± 0.5° C with a heat lamp regulated by a temperature controller (model 73 ATF, Yellow Springs Instruments, Yellow Springs, OH 45387) utilizing a rectal temperature probe. Arterial blood pressure was monitored with a miniature pressure transducer (Statham model P-37B, Gould Inc., Oxnard, CA 93030). Electrocardiographic activity (ECG) from the implanted electrodes, blood pressure, body temperature, and hydrogen-clearance curves were continuously recorded on a polygraph (Grass Instruments Co., Quincy, MA 02169). The ECG was subjected to serial frequency analysis on line by fast Fourier transform.

Air Embolization Technique

Before embolization the carotid catheter was flushed with heparinized saline. Air in 5-μl aliquots separated by saline was drawn into a 50-μl syringe (Gastight® #1705, Hamilton Company, Reno, NV 89510) mounted on a repeating dispenser (Hamilton). Once the hydrogen-clearance curve had descended beyond its halftime, an embolus of air was rapidly injected into the internal carotid artery. The ischemic effect of the air was assessed by the resulting changes in the ECG. The influence of blood pressure on air embolism was evaluated at a mean arterial blood pressure (MABP) of 100 Torr in 6 animals and 50 Torr in 5 animals. The blood pressure was regulated by withdrawing and infusing arterial blood. Acidosis during hypotension was corrected with intravenous sodium bicarbonate and adjustment of the ventilator.

[14C]-Iodoantipyrine Autoradiography

In three animals the distribution and degree of the ischemia were determined with an autoradiographic study of CBF by the method of Sakurada et al.25 Infusion of tracer was started within 30 sec after injecting a single 5-μl embolus of air.

Results

When injected during washout of hydrogen from the brain, air emboli of 5 μl caused an abrupt arrest or marked decrease of local CBF within the embolized cerebral hemisphere (fig. 2). In electrodes registering reduced CBF, the duration of the reduction (presumably representing the residence or transit time of the embolus)26 ranged from seconds to a couple of minutes at normal blood pressure (MABP of 126 ± 5 Torr). However, the alteration of CBF was not uniform across the array of electrodes: the lateral neocortex was most consistently affected for the longest time, the rostral thalamus and caudal hippocampus less so, and the medial neocortex least of all. This distribution pat-
TABLE 2  Regional Residence Times for a Single 5-μl Embolus of Air*

<table>
<thead>
<tr>
<th>Structure</th>
<th>50 Torr</th>
<th>100 Torr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rostral thalamus</td>
<td>0.67 ± 0.20</td>
<td>0.56 ± 0.26</td>
</tr>
<tr>
<td>Caudal hippocampus</td>
<td>1.02 ± 0.56</td>
<td>0.68 ± 0.34</td>
</tr>
<tr>
<td>Lateral neocortex</td>
<td>1.76 ± 1.00</td>
<td>1.04 ± 0.31</td>
</tr>
<tr>
<td>Medial neocortex</td>
<td>0.61 ± 0.25</td>
<td>0.22 ± 0.0</td>
</tr>
</tbody>
</table>

* Residence time is the duration in minutes (mean ± SEM) that CBF slowed at each site after embolization. The number of affected animals over the total number of animals in the group appears in parentheses. p > 0.05 between groups.

† One animal showed irreversible circulatory arrest and is excluded from the average.

tern occurred independently of blood pressure (table 2). Hypotension showed a tendency to increase the residence time of the embolus (table 2) and also the severity of ischemia as judged by more protracted suppression of the ECoG. Serial emboli usually produced a progressively more severe ischemic effect on the ECoG, despite the observation that the residence time of the air in the four monitored regions was not necessarily identical from embolus to embolus. Autoradiographic blood flow studies after embolism revealed a defect of perfusion in an area of brain varying from a large wedge in the distribution of the middle cerebral artery to the entire cerebral hemisphere including the ipsilateral midbrain (fig. 3).

Within 10–15 sec after injection of air the ipsilateral ECoG became attenuated or isoelectric. Electrical activity returned to normal if the interruption of flow was of brief duration (seconds), but, ordinarily, recovery of the amplitude of the ECoG was incomplete. Serial analysis of the frequency spectrum of the ECoG throughout ischemia disclosed loss of higher frequencies and an obvious shift of the predominant frequencies from 5–8 Hz down to 1–3 Hz. In 19 records analyzed, transient attenuation of the ECoG occurred in the contralateral, nonembolized hemisphere in 53% of animals and complete suppression in another 11%. No change in amplitude of the contralateral ECoG appeared in the remaining 37%. The CBF of the nonembolized hemisphere never manifested any abrupt decrease after injection of air: on the contrary, lateral neocortical electrodes occasionally detected increased flow after embolization.

During development of the model, two difficulties were soon recognized. The first was that the eyeball on the side of the embolization regularly blanched after injection of air. This pallor indicated loss of a portion of the embolus into the retinal circulation. To remedy the situation, exenteration of the orbit was incorporated into the model. A second problem that occurred in 5 or 10% of initial animals was the total lack of cerebral ischemia after repeated injection of air. In these cases it was discovered that the tip of the carotid catheter had entered the pterygopalatine artery, a major extracranial branch of the internal carotid artery. To obviate this difficulty it was essential to ligate the pterygopalatine artery as Johansson has previously advised. These two steps considerably enhanced the effectiveness and reproducibility of the model while also permitting the use of emboli as small as 2 μl.

Discussion

Gaseous emboli offer distinct advantages over solid emboli in models of cerebral ischemia. Intravascular bubbles induce neither the arterial vasospasm nor the hemorrhagic infarction so often encountered with solid material, except under special conditions. Moreover, the ischemia caused by intra-arterial gas is ordinarily reversible as the gas passes through the...
microcirculation under the force of the blood pulsations with or without the facilitation of a hyperbaric environment. If volumes of gas are delivered in a controlled and selective manner, then one can produce cerebral ischemia that is both transient and focal.

The catheterization scheme in the present model provides access to the internal carotid artery so that microliter volumes of air can be injected exclusively into the arterial supply of one cerebral hemisphere. Previously, Bidder has demonstrated that dye infused into one internal carotid artery of the rat remains confined to the ipsilateral cerebrum. To achieve this end, he passed a catheter into the internal carotid artery via the lingual branch of the external carotid artery. The anatomical approaches to catheterization by Bidder and the author are thus similar, and both preserve hemodynamic relationships within the brain. In the present model, moreover, diversion of air into noncerebral tissues was minimized by ligation of the pterygopalatine artery and by exenteration of the orbit.

Consonant with Bidder’s work, cerebral autoradiography revealed that air injected into the internal carotid artery remains within its territory of supply, chiefly within the distribution of the middle cerebral artery (fig. 3). After embolization, local CBF transiently fell to near zero in the ipsilateral hemisphere and usually remained unchanged in the contralateral hemisphere. Polarographic measurements of CBF confirmed the transient nature of the focal ischemia (table 2 and fig. 2). The duration of diminished flow varied among the four monitored regions of the cerebrum, and it tended to be lengthened by arterial hypotension (table 2). After a 5-μl embolus, ischemia was most consistently produced for the longest time in the lateral neocortex and least so in the medial neocortex which is perfused by the azygous anterior cerebral artery. Betweentheses two extremes lay the rostral thalamus and the caudal hippocampus. Presumably, the regional variation of residence time of the air embolus depends upon (1) the volume of gas entering upstream vessels supplying the neighborhood of the electrode and (2) the velocity with which the gas moves past the monitoring electrodes. If the velocity of the gas is considered comparable among various regions of cerebral gray, then differences in residence time can be attributed to unequal partitioning of the air embolus as it enters the distal ramifications of the internal carotid. Accordingly, the arterial supply of the lateral neocortex probably receives a greater fraction of the embolus than do deeper structures. This uneven distribution of the embolus and the resultant regional variation in the duration of ischemia may in part explain such questions as why cerebral capillaries are more permeable in the cortex than in the basal ganglia after air embolism and also why postembolic reperfusion of the brain is heterogeneous.

Finally, unilateral cerebral air embolism exerts indirect effects on the function of the contralateral hemisphere. Years ago, Swank and Hain observed suppression of EEG activity in both cerebral hemispheres after injection of solid emboli into one carotid artery. Nearly two-thirds of the animals in the present study also exhibited attenuation of ECoG function in the nonembolized hemisphere despite unchanged CBF. This phenomenon might be explained by a loss of normal “couple” between CBF and local metabolic demand, an apparent instance of diaschisis. Qualitatively, though, glucose consumption increases in the nonembolized hemisphere as shown by [%C]-2-deoxyglucose autoradiography. The physiological meaning of this rise in glucose utilization is unclear.

In conclusion, experimental air embolization is an attractive but deceptively simple means for producing cerebral ischemia. The method may serve as a model for the clinical syndrome of transient ischemic attack and specifically for investigation of the neurologic complications of decompression sickness or open-heart surgery. The model can also be applied to studies of the molecular interactions between air and the brain-blood barrier or between air and cerebral metabolism. Nevertheless, success in such endeavors requires that one be cognizant of the various idiosyncracies of the model in order to realize its maximal efficacy.

Acknowledgments

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References

Cerebral Ischemia Produced By Four-Vessel Occlusion in the Rat: A Quantitative Evaluation of Cerebral Blood Flow

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SUMMARY Cerebral ischemia was produced in the rat by simultaneous occlusion of the vertebral and carotid arteries according to the method of Pulsinelli and Brierley (Stroke 10: 267, 1979). Local cerebral blood flow (CBF) was determined by polarographic and autoradiographic techniques. Hydrogen-clearance measurements showed that mean CBF fell in four monitored regions of the hemispheres to between 0.11 and 0.18 ml/g/min, being least in deep rostral gray, intermediate in superficial gray, and greatest in deep caudal gray. However, individual animals had local CBF in excess of 0.20 and even 0.30 ml/g/min, and no animal showed zero CBF. When animals were rendered hypotensive (MABP of 50 Torr) during vascular occlusion, mean CBF ranged between 0.03 and 0.10 ml/g/min in the same regional order. With hypotension, total arrest of flow occurred. Autoradiographic data confirmed the above findings and indicated adequate CBF to the lower brainstem. During vascular occlusion, sufficient CBF may be present to sustain cerebral tissue as in animals with a well developed spinal circulation or an inadvertently patent vertebral artery.

THE MULTITUDE OF METHODS for producing cerebral ischemia in animals attests to the difficulty of imitating stroke in man. For instance, mere ligation of the carotids is ineffective in many animals including rat, owing to an ample collateral blood supply. Thus, the model of cerebral ischemia in the rat introduced by Pulsinelli and Brierley in 1979 was welcome. These investigators first electrocoagulated the vertebral arteries in the atlas and then occluded the carotid arteries. The experimental approach results in global ischemia of the cerebral hemispheres with relative sparing of the brainstem as assessed by dye perfusion and by neuropathologic changes. Owing to the lack of quantitative hemodynamic data in the original report, the following study was undertaken to determine the distribution and magnitude of reduced cerebral blood flow (CBF) in the Pulsinelli-Brierley model.

Materials and Methods

The method of Pulsinelli and Brierley was employed for producing experimental cerebral ischemia in male Sprague-Dawley rats (Charles River Farms) weighing 200–400 g. Under halothane-oxygen anesthesia a midline incision was made in the dorsal neck, and the cervical muscles were divided down to the atlanto-occipital junction. The alar foramina of the atlas were identified, and a monopolar microelectrode was passed into each foramen in turn. A current sufficient to cause slight muscle contraction was applied in order to electrocoagulate the underlying vertebral artery. The foramina were packed with bone wax, and the muscles and fascia were closed in layers. Through a ventral midcervical incision each carotid artery was
Experimental air embolism of the brain: an analysis of the technique in the rat.
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