A Modified Four-Vessel Occlusion Model for Inducing Incomplete Forebrain Ischemia in Rats

R. Schmidt-Kastner, MD, W. Paschen, PhD, B. Grosse Ophoff, MD, and K.-A. Hossmann, PhD

The four-vessel occlusion (4VO) model of Pulsinelli and Brierley (Stroke 1979; 10:267-272) has been modified for use in halothane-nitrous oxide–anesthetized, physiologically controlled rats that were ventilating spontaneously. Selection criteria for the classification of severity of ischemia were established by correlating changes in the electroencephalogram and the general physiological status with measurements of regional blood flow and regional energy metabolism. In 13% of animals, 4VO did not cause flattening of the electroencephalogram, and such animals were classified as undergoing only “oligemia.” In 65% of rats, the electroencephalogram flattened and blood pressure sharply increased with 4VO, whereas spontaneous respiration continued. This group exhibited almost complete ischemia in autoradiographic blood-flow studies, severe acidosis, and depletion of adenosine 5'-triphosphate and glucose in the forebrain and, hence, was classified as the “ischemia” group. The remaining 22% stopped breathing after vascular occlusion and were rejected for further study. Survival experiments of ischemic animals revealed the typical postischemic sequelae, with primary metabolic recovery after 8 hours of recirculation in all brain structures followed after 8–24 hours by severe biochemical deterioration and neuronal death in the striatum and hippocampus. Postischemic seizure activity was rare. The main advantages of the present modification in comparison with the original method are 1) the application of anesthesia without loss of primary selection criteria, 2) the possibility of invasive physiological monitoring, and 3) the absence of postischemic seizures, which eliminates the necessity for secondary selection criteria. (Stroke 1989;20:938-946)

In 1979, Pulsinelli and Brierley1 described a model of incomplete forebrain ischemia in unanesthetized rats that was produced by occlusion of four major arteries supplying the brain. Four-vessel occlusion (4VO) was performed in two stages: 1) by coagulation of both vertebral arteries through the alar foramen and 2) after a delay of 1 day, by transient occlusion of both common carotid arteries.1 The severity of ischemia is judged by neurological investigation, and animals are then selected for further study on this basis.1

Although the reliability of this model has been well established, the absence of anesthesia precludes application of invasive methods for the study of ischemic and postischemic pathophysiological changes. Use of anesthesia, on the other hand, eliminates the possibility of assessing the degree of ischemia by examining neurological signs. For this reason, we have established selection criteria of severe forebrain ischemia that can be applied to anesthetized, spontaneously breathing animals and that are compatible with postischemic survival.

Materials and Methods

We used 163 adult male Wistar rats of the Han-Wist strain (Zentralinstitut für Versuchstiere, Hannover, FRG) (mean body weight, 310 g). On the first day of the experiment, rats were anesthetized in a jar flushed with 4% halothane in 70% nitrous oxide-30% oxygen until unresponsive, placed on a rat operating table (Hugo Sachs, Freiburg, FRG), and maintained under anesthesia with a face mask surrounded by a larger, semiclosed container connected to an exhaust pump. This system allowed high flow of anesthetic gases combined with effective removal of expired carbon dioxide.2 Halothane concentrations (1.2–2.5%) were adjusted to achieve surgical anesthesia with the animal under spontaneous respiration. The head was mounted between two blunt flexible earbars and inflected anteriorly to
provide access to the occipital skull and upper vertebrae. Both vertebral arteries were cauterized with a monopolar coagulator (model t100, Aesculap, Tuttingen, FRG). After surgery, rats were kept in single cages, and food was withheld to stabilize plasma glucose levels.

On the following day, the rats were reanesthetized with 1.2–2.5% halothane in 70% nitrous oxide-30% oxygen by face mask, and the common carotid arteries were dissected free while being observed with an operating microscope. A small catheter was inserted 10 mm into the tail artery. The arterial line was connected to a blood-pressure transducer and was also used for blood sampling. Physiological parameters were recorded as described below. After termination of surgery, halothane was reduced to a maintenance dose of 0.5% and maintained in this state for 10–15 minutes until respiratory activity increased, hypercapnia weaned, and delta waves in the electroencephalogram (EEG) diminished. At this stage, the animals did not show signs of discomfort. Anesthesia was then discontinued, and the carotid arteries were occluded for 30 minutes by small, temporary clips (Biemer clips FD 562, Aesculap). Most rats remained unresponsive during ischemia, although a few animals exhibited signs of responsiveness and were immediately reanesthetized by a bolus of 4% halothane. During vascular occlusion, animals were kept in the recumbent position to yield a maximal stimulus for the righting reflex. After 30 minutes, the clips were removed, free flow through carotid arteries was ascertained by microscopic examination, and wounds were closed. Animals were returned to their cages with free access to tap water and standard rat chow. After different recirculation periods, animals were reanesthetized and the brains processed for biochemical analysis (at 0, 8, or 24 hours survival) or histological study (at 3, 8, 24, 48, or 72 hours) as described elsewhere.3-5

Blood pressure measurements in the small, tail-artery catheter were “dampened” and therefore handled as “mean” arterial blood pressure (MABP). Arterial blood samples of about 200 μl were taken before, during, and after ischemia and analyzed for PO2, Pco2, and pH in a blood-gas analyzer (Eschweiler, Kiel, FRG). Hematocrit and plasma glucose (glucose analyzer, Beckman Instruments, Arlington Heights, Illinois) concentrations were measured also. A rectal thermistor was inserted, and the temperature was maintained between 37.0°C and 37.5°C C with a feedback-controlled heating lamp. In addition, baseline heating of the operating table was provided to include the head of the rats. At the end of the experiment, relevant physiological parameters were remeasured (n=4–12 rats at 8, 24, or 72 hours). In experiments terminated under ventilation with anesthesia, blood gases were adjusted to adequate values (PO2=110–140 mm Hg, Pco2=37–40 mm Hg, and pH=7.39–7.47). In metabolic or blood-flow studies terminated during 4VO, physiological parameters were carefully kept in the same range as those in the main group during 4VO.

The EEG was used to monitor the depth of anesthesia during the preparatory phase, to assess the depth of ischemia at 4VO, and to examine the neurophysiological recovery after different survival periods (3 hours–3 days). For that purpose, silver needle electrodes 12 mm long were inserted bilaterally under the scalp, and the interhemispheric EEG was recorded on a strip-chart recorder (type RM Dynograph Recorder, Beckman Instruments). Electrocardiograms (ECGs) were also recorded to identify noncerebral signals during isoelectric periods.

The autoradiographic method described by Sakurada et al6 was used to determine regional cerebral blood flow (rCBF). Two animals used as controls underwent vertebral artery occlusion 1 day before. In four others, 4VO was induced, those with flat EEGs were selected (n=3), and rCBF was measured during the 29th minute of occlusion. A ramped infusion of [14C]iodoantipyrine was administered to control animals during a 30-second interval. Occluded rats received an infusion lasting 60 seconds to achieve high contrast in low-flow areas.7,8 Timed arterial blood samples were withdrawn for counting plasma radioactivity. After decapitation, brains were removed and frozen in cooled methylbutane, and 20-μm sections were cut in a cryostat. Mounted sections and a set of standards were exposed to X-ray film. Resulting autoradiograms were scanned on a rotating densitometer (Scandig 3, Joyce and Loebl, Gateshead, UK), and rCBF data were computed on a laboratory computer (pdp 11/24, Digital Equipment Corporation, Maynard, California). Autoradiograms were evaluated according to a standard atlas of the rat brain.9

For biochemical study, five groups of rats (n=6 each) were analyzed: intact controls, vertebral artery-occluded rats, animals during 4VO, and animals that had undergone recirculation for 8 or 24 hours after ischemia. Animals were ventilated with 1.0–1.5% halothane in 70% nitrous oxide-30% oxygen, and physiological parameters were stabilized. Then, the skull was frozen with liquid nitrogen with the funnel technique.10 Frozen brains were removed in a cold chamber and cut in a cryostat at −20°C. Coronal sections 20 μm thick from the level of the stratum were taken for imaging of tissue pH and adenosine 5'-triphosphate (ATP) distribution with fluorescent and bioluminescent techniques. In addition, tissue samples of about 10 mg were taken from the stratum, neocortex, hippocampus, and cerebellum. In these samples, ATP, phosphocreatine (PCr), glucose, and lactate content were measured by conventional fluorometric enzymatic techniques.11 For imaging tissue pH, brain sections were exposed to the fluorescent pH indicator umbelliferone and processed as described by Csiba et al.12 ATP bioluminescence was produced by the method of Kogure and Alonso,13 with modifications as described elsewhere.14
### TABLE 1. Physiological Parameters in Rats Before, During, and After Ischemia

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>4VO (n=64)</th>
<th>Recirculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 (n=17)</td>
<td>2 (n=64)</td>
<td>3 (n=15)</td>
</tr>
<tr>
<td>PO2 (mm Hg)</td>
<td>125±17</td>
<td>134±21</td>
<td>132±17</td>
</tr>
<tr>
<td>Pco2 (mm Hg)</td>
<td>49±4</td>
<td>45±7</td>
<td>47±5</td>
</tr>
<tr>
<td>pH</td>
<td>7.36±0.03</td>
<td>7.38±0.04</td>
<td>7.36±0.05</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>137±25</td>
<td>134±20</td>
<td>132±18</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>46±2</td>
<td>45±3</td>
<td>46±3</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>104±23</td>
<td>95±19</td>
<td>100±22</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>37.2±0.4</td>
<td>37.3±0.6</td>
<td>37.0±0.5</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD.

Groups of control measurements differentiate between animals that exhibited ischemia with respiratory arrest after 4VO (Group 1), ischemia after 4VO (Group 2), or oligemia after 4VO (Group 3). There were no significant differences among groups as tested for each parameter by ANOVA (p>0.05). All further data measured during 4VO and at different periods of recirculation apply to animals selected as ischemic and refer to group 2 for statistical reference (ANOVA, p<0.05 and t tests with correction for multiple comparison, p<0.05). Differences in the physiological status at 15 minutes during 4VO and that at 5 minutes of recirculation with regard to the control phase (Group 2) are indicated by †. Differences arising after 8, 24, and 72 hours of recirculation against the control (Group 2) by ‡. Note that since the latter animals were artificially ventilated, blood gases and pH were not tested.

#### Results

Experiments with 4VO were classified into three groups as shown in “Results.” To test for random occurrence of outcome, a trend analysis was performed with a run test and a phase-frequency test. All other data sets were tested by ANOVA (p<0.05) and modified t tests including the Bonferroni correction of critical significance. Data in text and tables are given as mean±standard deviation.

#### Results

One day after bilateral vertebral artery occlusion, 95% of rats were neurologically normal; only 5% of animals exhibited signs such as discrete unilateral limb weakness. Animals lost an average of 8% in body weight during this period. Physiological variables were measured in the preischemic stabilization phase to document the status of the anesthetized animals under spontaneous respiration (Table 1). Under delivery of 30% oxygen through the face-mask system, arterial PO2 was above 100 mm Hg. Due to narcotic hypoventilation, arterial Pco2 was slightly higher and pH slightly lower than in mechanically ventilated animals. Body temperature was about 37.3° C before the insult and remained constant. There were no statistically significant differences in any physiological parameters among the three different 4VO-outcome groups (see below) as tested by ANOVA. All animals were monitored by EEG recording during surgery and in the stabilization phase. During anesthesia with 1.2–2.5% halothane, the EEG was dominated by high-amplitude, slow waves. During the stabilization phase with 0.5% halothane, EEG amplitude decreased, and the frequency spectrum increased (Figure 1A).

After 4VO, some animals stopped breathing and were rejected for further study (22%). The other rats maintained spontaneous ventilation and were kept occluded for 30 minutes. Among these, two groups were distinguished according to the type of EEG changes. Eighty-three percent of the breathing rats (65% of total) revealed complete EEG flattening (Figure 1B) and were classified as the “ischemia” group. The other 17% of the breathing...
EEG recovery: 24 hours

FIGURE 2. Electroencephalographic (EEG) recovery recordings in three rats (A, B, and C) subjected to 30-minute four-vessel occlusion followed by 24 hours of recirculation. Recordings were performed under halothane-nitrous oxide anesthesia. Note the irregular EEG activity with low and sharp waves.

rats (13% of total) exhibited some persisting EEG activity (Figure 1C) and were classified as the "oligemia" group. Statistical analysis showed that the three patterns occurred at random (run test and phase-frequency test, p<0.05 in each test).

Physiological parameters in the ischemia group were measured again during occlusion and at 5 minutes of recirculation and tested against the control phase (Table 1). Slight hyperventilation occurred during 4VO (Pco2=35±9 mm Hg and pH=7.48±0.08). Plasma glucose or hematocrit levels changed slightly. MABP rose distinctly on 4VO by 54±21 mm Hg, remained elevated throughout 4VO (at 15 minutes of 4VO, MABP=140±34 mm Hg and at 28 minutes, MABP=146±27 mm Hg), then dropped to a minimum of 95±23 mm Hg on release of the clamps, and recovered to 115±21 mm Hg at 5 minutes of recirculation. Physiological parameters were retested in ischemic animals at different survival periods and compared with the physiological status before ischemia (Table 1). Plasma glucose content increased, whereas hematocrit levels remained constant. Blood pressures measured during halothane anesthesia were in the normal range. Body temperature was lower at 8 and 24 hours of recirculation than in controls but normalized toward 72 hours of survival. Loss in body weight amounted to 11% 1 day after ischemia, 18% after 2 days, and 22% after 3 days of recirculation.

Ischemic animals showing EEG suppression after 4VO remained recumbent and showed bilateral paw extension and only minor spontaneous movements. Their neurological status was classified as light coma, as judged from unresponsiveness to light, sound, and soft touch. In some animals, respiration stopped immediately after vascular occlusion but returned on mechanical stimulation of the thorax. Following reopening of the clamps, animals were positioned on one side where they remained in an unresponsive state for 1-3 hours. During this time, bilateral miosis was frequently observed. Then, the rats sat up and maintained a characteristic posture for the next 24 hours. The lumbar spine was hyperflexed, and the animals periodically searched the ground while chewing, biting, and moving the forepaws. These stereotypes could be interrupted immediately by acoustic stimuli, while visual responses were sluggish. Otherwise, there were no consistent neurological signs. By the 2nd day, posture and overall behavior tended to normalize, and most animals walked and ate or drank with some support (n=17 observations). Five percent of the breathing animals (n=6) died after the ischemic insult, four due to acute respiratory problems and only two in status epilepticus (see below).

Immediately after vascular occlusion in the ischemia group, EEG amplitude began to decrease, and some delta waves appeared until isoelectricity was seen after 15±3 seconds (Figure 1B). The EEG remained flat during the entire period of occlusion and during the initial 10 minutes of recirculation. Recovery of EEG was difficult to judge because halothane exerted a pronounced depressant effect after ischemia. Typically, by 8 hours of recirculation, high-amplitude bursts overlying slow, background activity were recorded. By 24 hours, irregular EEGs with slow and sharp wave activity were seen (Figure 2), and at 72 hours, continuous, fast EEG activity prevailed. Seizure activity was rare. Forty percent of animals showed sharp waves (Figure 2B) but never revealed a generalized pattern of epileptic activity. Before 24 hours of recirculation, no animal convulsed, and of those surviving 24 hours and longer (n=48), only two convulsed. Both these rats soon died in status epilepticus.

Results of regional cerebral blood flow are summarized in Table 2. Spontaneously breathing, control rats under light halothane anesthesia revealed depressed flow values in comparison with awake animals. Consequently, values of relative flow reduction under anesthesia underestimate the degree of ischemia in comparison with awake animals. Flow measurements were carried out in various brain areas at representative frontal section levels (-3.3, +4, +6, +8, and +10 mm with reference to the intra-aural line, in addition to sections at +5 mm for the hippocampus and +7 mm for the striatum). All values measured during 4VO were significantly different from controls (p<0.05). Most of the neocortex, striatum, and dorsal hippocampus had flow values below 6% of controls. However, gradients of
residual blood flow occurred along the ventrodorsal axis. In the neocortex, a clear decline of flow occurred from the basal cortex along the circumference toward the lateral, laterodorsal, and paramedian cortex (ANOVA, p<0.05). No differences arose along the rostrocaudal axis as tested in each subarea. Within the striatum, the ischemic effect was more severe in dorsal than in ventral areas (t test, p<0.001). In the hippocampus, regional differences in residual flow during occlusion failed to reach statistical significance.

In the ischemia group, a sequence of ATP-bioluminescence and pH-fluorescence pictures taken at the level of the striatum is shown in Figure 3.
FIGURE 3. Pictorial representations of adenosine 5'-triphosphate (ATP) (upper panel) and pH (lower panel) on cryostat sections of rat brains without ischemia (control), at the end of 30 minutes of four-vessel occlusion (ischemia), and after 8 (8h) and 24 hours (24h) of recirculation after 30 minutes of ischemia. ATP was measured by induced bioluminescence and pH with the fluorescent indicator umbelliferone (bright signals indicate high ATP and pH levels). Note the widespread depletion of ATP and acidosis during ischemia and the secondary decline of ATP in the striatum after 24 hours of recirculation.

During 4VO, ATP bioluminescence was absent in the forebrain except in circumscribed basal areas. At 8 hours of recirculation, ATP bioluminescence was homogeneous over the whole forebrain, but at 24 hours, loss of ATP bioluminescence became evident in the striatum. The pH-fluorescence pictures showed acidosis (low-to-absent fluorescence) during 4VO over the forebrain, which was followed by recovery at 8 and 24 hours of recirculation (high fluorescence). Quantitative data of pH changes in this model have been reported elsewhere. The results of enzymatic tissue analysis of energy metabolites in the neocortex, striatum, hippocampus, and cerebellum are shown in Table 3. Comparison of animals after vertebral artery occlusion with intact controls showed that the only significant change was in lactate values of the hippocampus, which were slightly higher in operated rats (controls=1.86±0.30, vertebral artery-occluded rats=2.36±0.42 μmol/g wet wt; t test, p<0.05). During 4VO, all forebrain structures exhibited massive depletion of ATP, PCr, and glucose, without differences between structures (ANOVA, p>0.05). Lactate levels rose to about 16 μmol/g wet wt in all forebrain regions, but higher levels were measured in the cerebellum, consistent with the observation that posterior brain structures received higher residual flows. After 8 hours of recirculation, ATP and PCR values had recovered in all structures, but lactate values remained elevated in the striatum. By 24 hours, energy metabolism deteriorated in the striatum as demonstrated by a further rise in lactate level and loss of ATP. In the hippocampus, lactate content also increased, but ATP and PCR values remained normal. Glucose content was increased in all areas at all recirculation times. In the neocortex of controls, the quotient (q) between brain and plasma glucose levels was 0.36±0.09. After ischemia, brain glucose levels increased more than plasma levels (q=0.50±0.08 at 8 hours [p<0.05] and q=0.52±0.04 at 24 hours [p<0.05]).

All animals classified as oligemic exhibited a physiological status before 4VO similar to that of the ischemic group (Table 1). During 4VO, two types of persisting EEG activity were observed (n=16 animals). In ten cases, low-voltage theta activity was recorded; these rats started to move and to right themselves despite previous anesthesia and had to be reanesthetized. The other six rats showed predominantly delta waves of high amplitude in their EEG records (Figure 1C). These rats remained calm and did not require reanesthesia. Because not all animals with persisting EEGs were able to right themselves from the recumbent position, this selection criterion was considered unreliable. Animals classified as oligemic awoke early after reversal of vascular occlusion and regained a normal neurological status during the 1st day after occlusion. After removal of the vessel clips, near-normal EEG activity quickly returned, and records obtained after survival periods for 1–7 days were indistinguishable from control tracings.

Discussion

This study introduced a modification of the 4VO model for producing cerebral ischemia in anesthetized, spontaneously ventilating rats. Several authors have made similar modifications of the 4VO model, with anesthesia or EEG as the selection criterion.
TABLE 3. Regional Energy Metabolism Before, During, and 8 and 24 Hours After 4VO Ischemia 

<table>
<thead>
<tr>
<th>After vertebral artery occlusion</th>
<th>During 4VO</th>
<th>During recirculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphocreatine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTX 3.78±1.31</td>
<td>0.22±0.53‡</td>
<td>5.11±0.46</td>
</tr>
<tr>
<td>HPC 3.54±1.67</td>
<td>0.15±0.36‡</td>
<td>3.49±0.45</td>
</tr>
<tr>
<td>STR 3.43±1.13</td>
<td>0.27±0.67‡</td>
<td>2.81±1.00</td>
</tr>
<tr>
<td>CB 5.48±1.45</td>
<td>0.20±0.48‡</td>
<td>5.18±1.53</td>
</tr>
<tr>
<td>Adenosine 5'-triphosphate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTX 3.04±0.50</td>
<td>0.06±0.05‡</td>
<td>2.77±0.36</td>
</tr>
<tr>
<td>HPC 2.18±0.22</td>
<td>0.14±0.21‡</td>
<td>2.08±0.68</td>
</tr>
<tr>
<td>STR 2.86±0.27</td>
<td>0.19±0.12‡</td>
<td>2.34±0.29</td>
</tr>
<tr>
<td>CB 2.75±0.52</td>
<td>0.49±0.29‡</td>
<td>2.70±0.55</td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTX 2.46±0.68</td>
<td>0.14±0.29‡</td>
<td>4.20±0.55†</td>
</tr>
<tr>
<td>HPC 1.79±0.63</td>
<td>0.13±0.15‡</td>
<td>3.12±0.48†</td>
</tr>
<tr>
<td>STR 1.52±0.51</td>
<td>0.10±0.20†</td>
<td>3.57±0.67‡</td>
</tr>
<tr>
<td>CB 2.47±0.61</td>
<td>0.07±0.11‡</td>
<td>3.72±0.75*</td>
</tr>
<tr>
<td>Lactate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTX 2.31±1.52</td>
<td>15.55±2.96§</td>
<td>1.53±1.11</td>
</tr>
<tr>
<td>HPC 2.36±0.42</td>
<td>16.36±1.93§</td>
<td>2.78±1.01</td>
</tr>
<tr>
<td>STR 2.29±0.75</td>
<td>15.16±1.61‡</td>
<td>7.02±2.74†</td>
</tr>
<tr>
<td>CB 1.89±0.72</td>
<td>20.36±6.07§</td>
<td>4.03±3.41</td>
</tr>
</tbody>
</table>

4VO, four-vessel occlusion; CTX, neocortex; HPC, hippocampus; STR, striatum; CB, cerebellum. All values are μmol/g wet wt (mean±SD), n=6 animals in each group. Statistical analysis was performed against the vertebral artery-occluded group (ANOVA, p<0.05, then modified t tests with *p<0.05; †, p<0.01; ‡p<0.001).

criterion. However, these studies did not examine how the modification influenced the selection of animals, as was required in the original method. In the present study, EEG recordings were used to differentiate two groups of animals. Animals with persisting EEGs obviously exhibited no critical degree of ischemia; they were classified as oligemic and excluded from further hemodynamic and metabolic analyses. Animals with flat EEG records were classified as ischemic. In these cases, the rCBF study, pictorial evaluation of ATP and pH, and quantitative measurements of energy metabolism documented a severe ischemic effect on the neocortex, dorsal hippocampus, and striatum. After ischemia, animals exhibited neurological deficits, pathological EEGs, and changes in energy metabolism. Previous histological studies in ischemic animals selected according to the present criteria revealed the typical pattern of ischemic injury in the hippocampus and striatum. The modified model, in consequence, closely resembles the original 4VO model in awake animals. However, halothane anesthesia might change adversely the physiological status in spontaneously ventilating animals. To keep the halothane dosage as low as possible, we added nitrous oxide as an analgesic agent. Influence of anesthetics was then tested by monitoring relevant physiological parameters. Before ischemia was induced, moderate hypercapnia was seen; however, this is unlikely to influence brain energy metabolism. Other variables such as arterial oxygen pressure, glucose, hematocrit, blood pressure, and body temperature were in the physiological range before the ischemic insult. The present model, in consequence, tends to stabilize rather than disturb the general physiological state of the animals. The physiological status of animals in the pres ischemic phase did not determine the outcome with 4VO, which suggests that intrinsic anatomic or physiological parameters were responsible for differential outcomes.

During occlusion of both vertebral and carotid arteries (4VO), some residual blood flow is supplied posteriorly from the anterior spinal artery, and forebrain flow will be reduced to a variable degree. In the original reports of 4VO, neurological signs were used to differentiate ischemic from nonischemic animals. In the present protocol, anesthesia precluded the reliable assessment of neurological signs. For this reason, the EEG was used as a selection criterion. According to this criterion, 65% of rats suffered forebrain ischemia below the threshold of EEG suppression. Thirteen percent had only...
a moderate reduction of flow still compatible with the generation of spontaneous electrical activity. In the remaining 22%, blood flow to the brainstem was critically reduced, and these animals died because of respiratory failure. These patterns occurred at random over time, and differences in preischemic physiological status did not explain the differential outcomes with 4VO. In the original report, 1 77% of animals were selected as ischemic on the basis of loss of the righting reflex, 15% were identified as having undergone only a moderate flow reduction, and 8% showed respiratory failure. The study of Steinberg et al.,27 which closely followed the original protocol, yielded 12% initial loss, and of the remaining animals, 87% did not right themselves. With EEG recordings, Combs and d'Alcay28 observed 79% of rats with isoelectric EEGs and 13% with incomplete suppression, whereas 8% had respiratory failure. Take et al.29 reported a loss of 20% with breathing arrest. This comparison demonstrates that the ratio of animals with ischemia and oligemia is rather similar, despite differences in anesthesia and selection criteria. Our series exhibited a higher percentage of respiratory failure, but a much lower incidence in generalized seizures, both of which phenomena are presumably related to anesthesia. In the original report, up to 40% of rats were excluded after ischemia because of convulsions.1 A recent study noted that animals with postischemic seizures had more pronounced histological damage.29 Therefore, it can be assumed that animals with seizures experienced more severe ischemia during 4VO. It is likely that addition of anesthetics in the present study exacerbated brainstem ischemia, and for this reason, more animals that otherwise might have experienced seizures died because of respiratory failure.

The pattern and density of rCBF changes in the modified 4VO model was in general agreement with previous studies.23,30,31 However, with conventional autoradiographic techniques, flow values below 10% of control are considered unreliable.7,8 In fact, the initial determinations of rCBF in the 4VO model have been criticized on these grounds.7 Therefore, the present study adapted the technique of long tracer infusion7,8 and succeeded in visualizing areas of residual flow more precisely. The general ventrodorsal organization of the major cerebral arteries in the rat25 suggests that, in low-flow states, ventral areas receive higher inflow than dorsal regions. In fact, it was noted that residual flow decreased from ventral to dorsal areas of the neocortex but did not decrease along the rostrocaudal axis, that is, between the territory of the three major cerebral arteries. In the striatum, ventrodorsal gradients of residual flow were also seen.

The biochemical measurements exhibited a rather uniform metabolic insult in all forebrain structures during 4VO, which was followed by a regionally heterogeneous recovery during recirculation. The postischemic deterioration of energy metabolism was more pronounced in our study than in previous reports of awake animals with 4VO and neck ligatures,24 but the localization of biochemical injury to the hippocampus and striatum was the same. The increase in lactate content observed at 8 hours in the striatum and at 24 hours in the dorsal hippocampus correlates with the appearance of histological lesions in these structures1,21 and reflects the beginning manifestation of neuronal death. The global increase in glucose content is not an indicator of damage but may result from changes in turnover as discussed by Arai et al.33

In conclusion, the described changes are very similar in the original and the modified 4VO model and confirm that anesthesia does not invalidate the usefulness of this model for ischemia research. The present modification, therefore, solves a major ethical problem of an otherwise most convenient experimental model without loss of reliability or changes in the general pathophysiological pattern. The described modification, in consequence, renders this model particularly suited for chronic experiments during which the animal should be exposed to a minimum of pain and discomfort during the ischemic phase.

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