A New Model for Inducing Transient Cerebral Ischemia and Subsequent Reperfusion in Rabbits Without Craniectomy

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An artificial ball removable by an attached fiber was injected into the middle cerebral artery (MCA) of 16 rabbits, allowing the study of transient (5, 10, 15, or 30 minutes) cerebral ischemia without craniectomy. Measurements of available oxygen ($\text{ao}_2$) in the ischemic core (the ventral part of the temporal area) followed by histologic examination verified the embolization. Electroencephalographic power spectra and steady (direct current) potentials were recorded bilaterally on the convexity remote from the actual lesion but still supplied by the MCA. Local cerebral blood flow and $\text{ao}_2$ in the border zone between the anterior cerebral artery and the occluded MCA were measured. Embolization caused typical ischemic changes ipsilaterally and alterations characteristic of diaschisis contralaterally. Extreme border zone hyperemia developed without significant $\text{ao}_2$ changes in the same region. Restoration of circulation via the circle of Willis induced gradual normalization. Our model for controlled embolization and recirculation proved suitable for detailed studies of the complex changes in brain function caused by transient ischemia. (Stroke 1988;19:1262-1266)

Despite great efforts to clarify the exact pathomechanism of cerebral ischemia, all pathophysiologic mechanisms involved are not yet known. For obvious reasons, it would be very important to establish the duration and extent of ischemia that is tolerated without irreversibly damaging the cerebral tissue. Despite its disadvantages, the most commonly used method for inducing experimental reversible cerebral ischemia is the transient clipping of the middle cerebral artery (MCA). We have developed a new method that allows transient occlusion of the MCA without craniectomy.

Materials and Methods

We used 16 New Zealand white rabbits of either sex (average body wt 2.5–3.0 kg) and 0.5 g/kg i.v. urethane with supplemental doses of intravenous sodium thiopentone as necessary for anesthesia. After one external carotid artery (ECA) was exposed, ligated, and transected at its first branching, the ECA was recurved and used to introduce a catheter into the internal carotid artery (ICA). The rabbits were then placed in a stereotactic head-holder and, while immobilized with 0.06 mg/kg pipercuronium bromatum, were artificially ventilated. Body temperature was maintained at 37° C. Lidocaine was used locally to ensure the pain-free condition of the rabbits. Electrodes and thermistors were inserted via 1-mm-diameter burr holes on both sides of the skull (Figure 1).

Injection of silver ball emboli into the ICA reliably occludes the ipsilateral MCA. Initially, a silver ball 0.40–0.45 mm in diameter soldered to a fine copper wire and later, the same size balls made of glue (Krazy Glue, Inc., Itasca, Japan) attached to a strong, thin, flexible fiber (Ethicon, 8-0 Monofil), were injected into the MCA via the ICA. After it was painted with nail polish to enhance visibility and connected to one end of the fiber, the ball was placed within the tip of a thin cannula (o.d. 1 mm) (Figure 2). The rest of the fiber was pulled through this first catheter and coiled within the lumen of a larger catheter, which was attached to a syringe. The outer diameter of the second catheter was 1.55 mm, and it could slide onto the end of the first catheter. The terminal portion of the fiber ran between the two adjacent catheter walls. The loose end of the fiber was held in place by the tightly appressed catheters in such a way that it controlled the length of the coiled portion. This also allowed quick withdrawal of the ball after it had moved forward by simply removing the larger catheter and pulling on the fiber. To prevent local thrombosis and/or air embolization, both catheters were filled with heparinized saline...
before insertion. The ball was directed into the MCA by inserting the thinner catheter into the ICA via the ECA (Figure 2) and injecting 0.2 ml saline from the syringe. The common carotid artery (CCA) was clipped during the entire experiment. The ECA, together with the thinner catheter, was occluded by a clip just after withdrawal of the ball.

The appropriate length of fiber was determined in a separate set of experiments by postmortem examination of six rabbits. Our initial trials proved that, by setting the length of the coiled portion of the fiber at 4.5 cm, we invariably could occlude the main trunk of the MCA. The embolus reproducibly localized there because balls with a diameter of >0.40 mm could not enter the branches of the MCA. In our preliminary experiments, typical changes of the electrical activity of the brain (EEG) ascertained that embolization had occurred.

A more detailed study of the pathophysiologic consequences of transient MCA occlusion was carried out on four groups of four rabbits each. The larger catheter was removed 5, 10, 15, or 30 minutes after embolization. Withdrawal of the ball was followed by 2 hours of recirculation via the circle of Willis. After the experiments, the brains were routinely processed for light microscopy.

Blood pressure (BP) was measured with a catheter placed in the abdominal aorta via one femoral artery. Arterial blood was sampled before and several times after embolization (including during recirculation) to ascertain that blood gases (Pao2, Paco2), pH, and blood glucose concentration were within the physiologic range. To verify embolization, available oxygen (aO2) was continuously measured using 75-μm-diameter open-tip gold wire electrodes (polarographic method) in the ipsilateral ventral-temporal cortex, where the most severe ischemic lesion was induced. EEG was recorded bilaterally with 4-6 gold ball dural electrodes within the MCA territory (Figure 1). The reference electrode was placed in the midline of the nasal bone. Monopolar derivation of the EEG was used. Percentile participation of the beta, alpha, theta, and delta frequency bands in the total electroencephalographic power spectrum (EEG-PS, μV²/s) of two bilateral, homologous middle temporal EEG record-
ings were computed (see Figure 1, Electrodes 3 and 7). Steady (direct current) potentials (DCPs) were recorded with the EEG electrodes.

Local cerebral blood flow (LCBF) and local cortical \( a_o \) on the convexity were measured in the border zone supplied by the MCA and the ACA (Figure 1). To avoid artificial lesions, thermistors were intentionally not placed in the ischemic core itself. LCBF was measured by the heat clearance method. Two thermistors were placed on the cortex; one was heated while the other served as a reference (Figure 1). Details of calibration and linearity of the technique over a wide range of changes have been discussed. \(^{9-12}\)

The data (BP, \( a_o \), EEG, DCPs, LCBF) were digitized by an analog-to-digital converter (sampling frequency 80/sec, integration every 3 seconds), stored on magnetic tape, and analyzed off-line with a computer. For statistical analysis, we used Student's \( t \) test or Welch's \( D \) test. We defined significant changes (\( p<0.01 \)) in individual rabbits by comparison with starting values (the average of 3-minute baseline recordings).

**Results**

The effects of embolization and ischemia of various durations on the percentile changes of ipsilateral \( a_o \), EEG-PS, DCPs, and LCBF are shown in Figure 3. The depression of total EEG-PS was caused by decrease of the percentile participation of the delta and theta bands and increase of the alpha band. All changes were significant except for the beta band and the cortical \( a_o \) on the convexity.

The effects of ischemia in the ipsilateral hemisphere after recirculation are presented in Figure 4. On the contralateral side, embolization always induced an immediate and significant decrease of total EEG-PS (35 ± 10%), and the theta band became dominant. The DCP changes were inconsistent.

Figure 5 shows a recording of these parameters in both hemispheres during 5 minutes of ischemia and during subsequent recirculation.

On the contralateral side, reperfusion after 5 minutes of ischemia promptly induced a transient and significant increase of total EEG-PS (30 ± 8%). After 10 and 15 minutes of ischemia, total EEG-PS increased significantly within the first 25 minutes of recirculation. After 30 minutes of ischemia, total EEG-PS decreased significantly before it normalized. DCPs showed changes similar to those in the ipsilateral hemisphere. Normalization in the contralateral side required 5–6 minutes of recirculation after 5 minutes of ischemia, 60 minutes after 10 and 15 minutes of ischemia, and approximately 90 minutes after 30 minutes of ischemia.

Histologic investigation revealed no definite cellular changes in the brains of rabbits embolized for 5–15 minutes. In the temporal areas, hyperemia and edema were the most characteristic microscopic features and were confined mainly to the cortex. The degree of pathologic changes depended on the duration of ischemia; after 30 minutes of ischemia, recirculation failed to prevent hypoxic cellular changes verifiable by light microscopy, namely, shrinkage and eosinophilic homogenization of the neurons.

**Discussion**

We had previously described a method of embolization of the MCA that circumvented the disadvantages of surgery but did not permit reopening of the occluded artery. \(^{6}\) Our current technique examines the effects of ischemia of defined duration and subsequent reperfusion without craniectomy. We used rabbits because their cerebral vasculature resembles that of humans (i.e., the ICA is the main artery supplying the brain\(^{13,14}\)).

The decreased \( a_o \) in the depths of the temporal area proves that embolization was successful since, after obstruction of the main trunk of the MCA, the ventral part of the temporal area was the most severely damaged. \(^{6}\)

The effects of embolization and ischemia that we observed agree with data from the literature and include the phenomena of increased perfusion and oxygen supply\(^{15-18}\) as well as reversible edema\(^{19,20}\) in the areas surrounding the ischemic core and diaschisis. \(^{21}\)

In the border zone on the convexity, \( a_o \) did not change significantly and was independent of the dura-
tion of ischemia. The stability of $ao_2$ with synchronously increasing LCBF in the same region reflected increased oxygen demand during occlusion of the MCA. Five minutes of ischemia did not result in functional cerebral damage because recirculation promptly caused gradual normalization of $ao_2$. We cannot explain the transient increase in total EEG-PS during recirculation after 5 minutes of ischemia.

Although the number of experiments is relatively small, it seems justifiable to state that in circumscribed transient cerebral ischemia there is a transient change of various physiologic parameters in the entire brain. The time for normalization depends on both the duration and the side of the occlusion of the MCA. Characterization of the relations between changes in the parameters described above requires further investigation. Our model is suitable for this purpose.

References
FIGURE 5. Right-sided embolization of rabbit brain. Effect of 5 minutes of ischemia followed by recirculation on local cerebral blood flow (LCBF) in border zone and on available oxygen in ischemic core (T\textsubscript{aO\textsubscript{2}}). Bilateral changes of electroencephalographic power spectra (EEG-PS) and direct current potentials (DCPs) are also shown. *\(\alpha\), periods of significant changes (\(p<0.01\)) compared with starting values defined as average of 3-minute baseline recordings. *\(\alpha\), either positive shift of DCPs or increase of other variables; \(\alpha\), either negative shift of DCPs or decrease of other variables.


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