Platelet Embolism in Rabbit Brain

STEFANO PASSERO, B.S., NOE BATTISTINI, M.D., AND CESARE FIESCHI, M.D.

SUMMARY  Transient cerebral ischemia was induced in rabbits by selective infusion of arachidonic acid (0.35 mg/kg in 15 sec) into the internal carotid artery. Platelet embolism caused transient ischemia of the brain, reaching a maximum within a few seconds after injection. After embolism the EEG flattened, blood flow stopped in almost the entire injected hemisphere, cortical pH gradually fell from 7.31 ± 0.09 to 7.05 ± 0.10 and cortical K⁺ activity rose from 4.7 ± 1.8 to 12.7 ± 6.4 mmol/kg H₂O. Complete ischemia lasted 3-5 min; then cerebral circulation was gradually restored without reactive hyperemia. Forty-five min after embolization, circulation had been resumed in almost the entire injected hemisphere, whereas metabolic changes were still disturbed. Eighty percent of the animals recovered complete neurological function and 20% showed permanent damage confirmed by histological examination after 1 week of recovery.

SEVERAL INVESTIGATIONS have given evidence that the brain has a capacity to recover both metabolic and neurophysiological functions following an extremely long period of ischemia.1-4 In contrast, clinical experience as well as a number of experimental studies have shown that following, for example, cardiac arrest, signs of irreversible brain damage may develop even if the primary ischemia only lasts a few minutes.9 The ultimate recovery of the brain following transient ischemia is not only dependent on the duration and intensity of the ischemia, but also on pathophysiological events during post-ischemia.

To interpret the mechanisms responsible for the final sequelae of ischemia, clarification of early metabolic and functional alterations in affected brain tissue is of considerable importance.

One type of cerebral ischemia of clinical importance is focal cerebral transient attacks (TIA) because they indicate increased risk for subsequent development of cerebral infarction. In the present series of experiments we have studied the changes in regional cerebral blood flow (rCBF) and metabolism in the rabbit brain during the post-ischemic period following a transient ischemic attack mimicked by producing intravascular platelet aggregates with selective intracarotid injection of arachidonic acid.

Material and Methods

The experiments were performed on 38 adult rabbits weighing between 1.4 and 2.0 kg. All animals were anesthetized with sodium pentobarbital (30 mg/kg i.v.). A tracheotomy was performed and respiration was controlled with a Harvard respirator pump using gallamine triethiodide. End-tidal carbon dioxide content was continuously monitored with a Godard infrared CO₂ analyzer.

Arachidonic acid (99% pure, Sigma USA) was dissolved in a nitrogen atmosphere at 0°C in saline solution at a concentration of 2 mg/ml. Aliquots of 0.5 ml of the resulting sodium arachidonate solution were pipetted into glass ampules, frozen in liquid nitrogen and sealed under vacuum. All ampules were stored in the dark at -60°C until use. Just before use the ampules were warmed in a water bath at 37°C and the desired amount was taken for injection.

Local H⁺ and K⁺ was recorded continuously by microelectrodes in the perivascular space of pial arteries. The pH electrode was the bulb type with a tip diameter of approximately 50 μm. The potassium electrodes, with a tip diameter ranging from 2 to 5 μm, were filled with Corning potassium exchanger as originally described by Walker.18 An Ag-AgCl electrode was used as a reference electrode. The signals were recorded across the differential input of preamplifiers with high input impedance (10¹⁰ ohm).

Local CBF was measured by the 14C-antipyrine autoradiographic method.14 In 5 animals a 40 sec intravenous antipyrine infusion was started immediately after the arachidonic acid intra-carotid injection. In the remaining animals the antipyrine infusion was started 15 min (n = 6) and 45 min (n = 6) after arachidonic acid injection. Animals were decapitated at the end of the procedure, the brain rapidly removed, frozen at -60°C then sectioned. Coronal sections for subsequent autoradiographic evaluation of local tissue concentration of antipyrine were sampled every 0.3 mm. The full extent of areas of complete ischemia was measured with a planimeter on magnified autoradio-
Extent and duration of embolism was followed by vital microscopy of the cortical surface and microphotographs were made before and at different times after arachidonic acid injection.

Results

End tidal CO₂ content and arterial blood gases were not significantly affected by the experimental procedures. Systemic arterial pressure decreased 11.5% during arachidonic acid injection from 95.6 ± 13.9 to 87.3 ± 13.5 mm Hg (n = 25). It returned to the baseline value in the subsequent 5-6 min and remained practically unchanged during the experiment.

Platelet Embolism and Recirculation

The time course of platelet embolism was studied by vital microscopy of pial vasculature. Within a few seconds after arachidonic acid injection blood flow stopped in the cortical vessels. The arrest of cerebral circulation in the injected hemisphere was due to transient embolization of the ipsilateral portion of the circle of Willis, as shown by the postmortem findings in animals killed immediately after arachidonic acid injection (n = 5). Complete ischemia lasted 3-5 min thereafter. Cerebral circulation was gradually restored and almost complete recovery occurred within an hour. During this time microscopic observation of the cortical vessels invariably showed macroaggregates moving into the large vessels; some of them were arrested temporarily at arterial bifurcations, then fragmented and gradually passed into veins (n = 12) (fig. 1). After arachidonic acid injection, 80% of the animals recovered complete neurological function and only 20% show permanent focal cerebral damage confirmed by histological examination performed after 1 week of recovery (n = 5).

Cerebral Blood Flow and Ischemia

Immediately after embolism, large areas of complete ischemia were observed in all animals involving on average 80.4 ± 11.4% of the whole hemisphere, from 64.6 ± 6.7 in section 1 to 90.8 ± 7.5 in section 4 (n = 5) (fig. 2). Ischemic areas were not predominant in any particular arterial territory, the entire injected hemisphere being involved. In some instances small areas of ischemia were also found in the territory of the contralateral anterior cerebral artery. Local blood flow in the areas not affected by complete ischemia showed a marked decrease in all structures considered (table).

After embolization blood flow gradually recovered and areas of complete ischemia decreased to a mean value of 36.0 ± 5.9% after 15 min (n = 6), and to 13.6 ± 3.9% after 45 min (n = 6) (fig. 2).

In no instance were focal areas of reactive
TABLE Local Cerebral Blood Flow After Arachidonic Acid Intracarotid Injection (0.35 mg/kg in 15 sec)

<table>
<thead>
<tr>
<th>Structure</th>
<th>Control hemisphere</th>
<th>Injected hemisphere</th>
<th>% of control</th>
<th>Control hemisphere</th>
<th>Injected hemisphere</th>
<th>% of control</th>
<th>Control hemisphere</th>
<th>Injected hemisphere</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 seconds (n = 5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>58.0 ± 4.2</td>
<td>0.0</td>
<td>0.0</td>
<td>56.4 ± 4.6</td>
<td>44.4 ± 7.4</td>
<td>78.7*</td>
<td>60.1 ± 4.2</td>
<td>49.0 ± 7.2</td>
<td>81.5</td>
</tr>
<tr>
<td>Basal ganglia</td>
<td>55.3 ± 3.8</td>
<td>0.0</td>
<td>0.0</td>
<td>58.5 ± 3.5</td>
<td>45.7 ± 4.8</td>
<td>78.1*</td>
<td>54.5 ± 5.1</td>
<td>48.5 ± 6.8</td>
<td>89.0</td>
</tr>
<tr>
<td>Cortex (mean of 4 structures)</td>
<td>57.5 ± 5.1</td>
<td>23.8 ± 5.3</td>
<td>41.4**</td>
<td>59.2 ± 4.8</td>
<td>32.4 ± 4.5</td>
<td>54.7**</td>
<td>58.5 ± 5.3</td>
<td>50.0 ± 6.5</td>
<td>84.5</td>
</tr>
<tr>
<td>Thalamus (mean of 6 structures)</td>
<td>55.0 ± 3.3</td>
<td>31.0 ± 4.7</td>
<td>54.5**</td>
<td>53.3 ± 2.3</td>
<td>35.2 ± 2.4</td>
<td>66.0**</td>
<td>50.0 ± 2.8</td>
<td>42.5 ± 3.2</td>
<td>85.0</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>35.0 ± 3.1</td>
<td>0.0</td>
<td>0.0</td>
<td>35.8 ± 2.8</td>
<td>23.9 ± 3.0</td>
<td>66.7*</td>
<td>32.6 ± 3.0</td>
<td>30.3 ± 3.6</td>
<td>92.9</td>
</tr>
<tr>
<td>White matter</td>
<td>18.5 ± 2.0</td>
<td>7.5 ± 1.8</td>
<td>40.5**</td>
<td>17.1 ± 1.9</td>
<td>15.5 ± 2.8</td>
<td>90.6</td>
<td>21.5 ± 2.3</td>
<td>20.9 ± 3.1</td>
<td>97.2</td>
</tr>
</tbody>
</table>

*p < 0.05.
**p < 0.002.

hyperemia (luxury perfusion) observed although in similar experimental conditions a significant increase of glucose utilization was measured with the autoradiographic technique employing 14C-deoxyglucose (fig. 3).

EEG

Electroencephalographic evidence of acute cerebral injury regularly appeared in all animals (n = 20) after arachidonic acid injection. Within 10 sec after beginning injection the amplitude of the background wave pattern attenuated, and diffuse, slow (2-4 c/s) activity appeared over the ipsilateral hemisphere. By 25 sec pronounced suppression of electrical activity became evident. Over the same time period, the contralateral hemisphere showed amplitude attenuation but fast activity was preserved (fig. 4). Flattening of the EEG lasted 3-5 min thereafter; then electrical activity was gradually restored.

Cortical pH and K+ Concentration

After arachidonic acid injection, extracellular pH was gradually reduced from 7.31 ± 0.09 to 7.05 ± 0.10 (15 min), then began to recover, reaching a mean value of 7.11 at 30 min and 7.15 at 45 min (n = 4).

Local K+ increased progressively from 4.7 ± 1.8 mmol/kg H2O to 12.7 ± 6.4 mmol/kg H2O (15 min) with a slight trend toward recovery in the subsequent time period (n = 4) (fig. 5).

Discussion

The clinical counterpart to the present experimental situation is a protracted transient ischemic attack, or a stroke with incomplete recovery, depending on the time and completeness of recirculation.19, 20 There is good evidence that most TIA's are due to embolism of the cerebrovascular bed with blood constituents. Current evidence suggests that platelets play an important role in the pathogenesis of arterial thrombosis.21

Transient cerebral ischemia has been studied by producing intravascular platelet aggregates with adenosine diphosphate,10, 14 by producing embolism of the capillary bed with microspheres, by arterial air embolism, or by temporary interruption of blood supply through clamping extracerebral or intracerebral vessels.22-27 These experimental models present some limitations or side effects. ADP-induced embolism causes peripheral effects resulting in severe hypotension. Microsphere embolism is usually fatal, is restricted in usefulness to acute experiments and has no counterpart in man. Intracerebral arterial occlusion requires craniectomy and extra cerebral arterial occlusion requires adding hypotension or hypoxemia that may affect other organs in addition to the brain.

Arachidonic acid-induced platelet embolism has few side effects and limitations. Along with the model of transient occlusion of the internal carotid artery in awake rats, pretreated with basilar artery occlusion,28 this method may be a better experimental means to produce reversible cerebral ischemia.
Studies of the cerebral circulation immediately following ischemia have demonstrated several possible patterns of recovery. Recovery may be incomplete due to localized or confluent areas in which perfusion fails to be resumed ("no reflow"). Other studies have shown no immediate impairment of reperfusion, or hyperperfusion.

The failure of resumption of flow in the brain has been attributed to a combination of factors such as the occurrence of intravascular aggregation of red cells and increased viscosity due to the stasis of blood during ischemia. High arterial pressure is an important secondary factor in the etiology of the hyperemia. Post-ischemic hyperemia is generally followed by hypoperfusion due to the development of vasogenic brain edema that may lead to progressive deterioration of cerebral perfusion until irreversible functional damage occurs.

With arachidonic acid-induced platelet embolism, cerebral ischemia was followed by a resumption of circulation 45 min after embolization and circulation was resumed in almost the entire injected hemisphere, so that 80% of the animals recover complete neurological function and 20% show permanent focal cerebral damage.

In no instance were areas of reactive hyperemia observed in spite of the presence of a severe cerebral tissue acidosis and, as reported by Fieschi et al., a significant increase in cerebral glucose utilization. The absence of hyperemia can be explained by the fact that the post-ischemic arterial pressure in all the animals in the present experiments fell in the low-normal range.
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There is good reason to believe that post-embolic recovery of cerebral blood flow observed in our experimental model may be the result of 1) the absence of a prolonged stasis of blood in the cerebral vessels; 2) the fact that emboli continue to move to different positions within the microcirculation in the course of the experiment; 3) the possibility that emboli do not cause complete occlusion of the vessel lumen; and 4) absence of post-ischemic hyperemia with consequent development of vasogenic brain edema and high intracranial pressure.

Two important points are relevant to the pathophysiology of cerebral ischemia: 1) While high perfusion pressure seems to be necessary to reinstitute blood flow after a period of ischemia due to temporary stasis, in ischemia due to thromboembolism reestablishment of blood flow takes place even if the perfusion pressure fell in the low-normal range; and 2) post-ischemic reactive hyperemia is not a constant pathophysiological consequence of transient brain ischemia but becomes common only in the presence of a severe arterial hypertension.

It follows that in the treatment of an ischemic episode, a brief period of induced hypertension may be a rational measure to promote blood flow in ischemic areas only when ischemia is due to temporary stasis.

References

34. Lassen NA: The luxury perfusion syndrome and its possible relations to acute metabolic acidosis localized within the brain. Lancet 2: 1113-1115, 1966
Role of Prostaglandin I$_2$ and Thromboxane A$_2$ in Recurring Reduction of Carotid and Cerebral Blood Flow in Dogs

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SUMMARY The roles of PGI$_2$ and TXA$_2$ in recurring reduction of carotid artery and cerebral blood flow induced by partial constriction of the common carotid artery were examined in anesthetized dogs. The recurring reduction was eliminated by OKY 046 and 1580 which inhibit TX synthetase, acetylsalicylic acid which inhibits cyclo-oxygenase and lipoxygenase, PGI$_2$, and by papaverine which enhances PGI$_2$ synthesis. But the recurring reduction was not eliminated by pentolamine. The recurring reduction was induced by epinephrine which activates phospholipase A$_2$ and cyclo-oxygenase and causes platelet aggregation. It was also induced by tranylcypromine which inhibits PGI$_2$ synthetase and, although infrequently, by TXA$_2$. The recurring reduction was also induced by indomethacin that inhibits cyclo-oxygenase. The indomethacin-induced recurring reduction, however, was eliminated not by OKY 046 and 1580 but by PGI$_2$. It is suggested that TXA$_2$ acted as an inducer and PGI$_2$ as an inhibitor in the recurring reduction of carotid artery and cerebral blood flow.

DURING partial constriction of the canine common carotid artery, there are frequent recurring decreases of a transient nature in the blood flow in the constricted artery and in the cerebrum. Participation of vasospasm, platelet aggregation, thrombus formation and embolism in this recurring CBF reduction has been demonstrated angiographically and histologically. This experimental phenomenon resembles transient focal cerebral ischemic attacks in that cerebral artery flow reduction occurs spontaneously and recurrently in both. It is not known whether thromboxane A$_2$, which causes platelet aggregation and contraction of vascular smooth muscles, and prostaglandin I$_2$, which inhibits platelet aggregation and relaxes vascular smooth muscles, participate in this experimental phenomenon. This study was undertaken to clarify the roles of thromboxane A$_2$ and prostaglandin I$_2$ in the recurring reduction of carotid artery and cerebral blood flow induced by partial constriction of the canine common carotid artery.

Methods

1. Experimental Preparations

Ninety-five beagle dogs were anesthetized with intravenous pentobarbital sodium (35–40 mg/kg). In each dog the trachea was intubated for artificial positive pressure respiration with air. The left common carotid artery was dissected free and a magnetic flowmeter (Nihonkoden MF-2) was placed around it to measure carotid artery blood flow. The superior thyroidal artery was cannulated to measure carotid blood pressure. A segment of the common carotid artery, 2–3 cm proximal to the thyroidal artery but distal to the flowmeter, was constricted with a cylindrical tube 3 mm in length and of various internal diameters. Usually, the blood flow was reduced by constriction to 30 to 70 percent of the control value. A pair of needle type cross-thermocouples were introduced into the anterior or lateral lobe of the cerebrum for measurement of cerebral blood flow. A catheter was introduced into the right femoral artery to monitor the systemic blood pressure. Heart rate was obtained by a pulse-integrator triggered by the femoral arterial pulse. A catheter was introduced into the brachiocephalic artery with the tip at the orifice of the left common carotid artery for selective injection of the chemical agents (fig. 1). After the experiments, ventricular fibrillation was induced by electrical stimulation. Following the stimulation, the
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