CEREBRAL ISCHEMIA is known to cause rapid changes in brain chemistry and function. When circulatory arrest occurs, brain energy reserves quickly become depleted, neuronal membranes depolarize, extracellular fluid shifts into the intracellular compartment and neuroelectric activity ceases. Ischemia also stimulates the activity of cellular lipases, whose actions cause deacylation of brain phospholipids and release of free fatty acids.1,2 Arachidonic acid (A.A.) is one of the predominant fatty acids released in brain after ischemia.3-6 A.A. is the primary substrate for the synthesis of prostaglandins, thromboxanes and prostacyclin. The availability of non-esterified A.A. is generally thought to be the rate limiting factor in the production of most prostaglandins.6 Only small changes in the levels of prostaglandins in brain occur after interruption of the cerebral circulation in gerbils following bilateral common carotid occlusion.7,8 Most likely, depletion of tissue oxygen in severely ischemic brain limits A.A. conversion to PG endoperoxides by cyclooxygenase, a step requiring the addition of molecular oxygen.6

Previously we reported that reperfusion of the brain, after episodes of brief ischemia, resulted in a large accumulation of A.A. metabolites in brain tissue.3 Presumably, re-establishment of blood flow restores tissue oxygen and permits the conversion of A.A. released during ischemia. We suggested that “non-total” ischemia can promote A.A. metabolism if residual flow provides enough O_2 for enzymatic conversion to endoperoxides. Furthermore, A.A. could diffuse from areas of focal ischemia to non-ischemic areas and be metabolized. These considerations could explain the elevated levels of PGF_2alpha and PGE_2 found in spinal fluid of patients with stroke.5,7

The effect of unilateral common carotid artery occlusion (CCA) occlusion on the levels of prostaglandins D_2, F_2alpha and 6-Keto-Prostaglandin F_1alpha in gerbil brain

ROBERT J. GAUDET, PH.D. AND LAWRENCE LEVINE, SC.D.

SUMMARY The concentrations of the arachidonlc acid metabolites, PGD_2, PGF_2alpha, and 6-keto-PGF_1alpha, were measured in the cerebral hemispheres of gerbils subjected to unilateral carotid occlusion. Approximately 37 percent of the animals with occlusion had symptoms of cerebral ischemia. In those animals PGD_2 and PGF_2alpha levels were elevated in both hemispheres. The levels of 6-keto-PGF_1alpha increased only slightly. There was no change of prostaglandin levels in asymptomatic animals. Indomethacin inhibited the increase in the levels of these arachidonic acid metabolites but did not alter brain swelling as judged by a decrease in specific gravity after 6 hours occlusion.

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causes cerebral ischemia in only 40% of gerbils because of an incomplete and variable circle of Willis.\textsuperscript{15, 16} In most of the animals suffering ischemia, there is usually some residual flow,\textsuperscript{18} which is probably derived from the opposite carotid artery by way of an anterior communicating artery. The use of unilateral CCA occlusion, therefore, allows investigation of biochemical changes in areas of brain subjected to "nontotal" ischemia and in remote areas (contralateral hemispheres) which are not ischemic. There is considerable evidence that A.A. metabolites as well as A.A. and other fatty acids are capable of potentiating inflammation in injured tissue.\textsuperscript{20-22} This prompted study of the effect of an inhibitor of PG synthesis (indomethacin) on the development of edema in ischemic gerbil brain.

Materials and Methods

Adult male and female Mongolian gerbils (Meriones unguiculatus) weighing 40 to 55 grams (West Jersey Biological Farms, Wenonah, NJ) were housed in simulated day and night conditions and allowed free access to food and water. In each animal the right common carotid artery was isolated and doubly ligated with suture. The operation required only a small incision in the neck and was performed using ether anesthesia.

Animals were judged "symptomatic" or "asymptomatic" depending on the appearance of clinical signs of ischemia. These signs included lethargy, circling, abnormal posturing, ptosis and running-jumping fits. Fits were sometimes followed by a period of muscle hypertonicity characterized by tonic limb hyperextension. Animals often died during these fits, apparently as a result of respiratory arrest during the tonic phase. To lessen the number of deaths, animals were sometimes given artificial respiration. This was done by alternately pressing the animal's rib cage, then blowing air into the lungs using rubber tubing which covered the animal's nose and mouth.

Groups of 5 to 7 animals were sacrificed under liquid nitrogen at 15 minutes, 2 hours and 6 hours after occlusion. Sham operated animals, in which the right CCA was exposed but not occluded, were sacrificed at the same time intervals after operation for controls.

The cerebral hemispheres were removed while still frozen, and the brain stem and prominent venous sinuses trimmed off. Each hemisphere was weighed and homogenized in 2.5 ml of 100% ethanol using a high speed tissue homogenizer. The samples were centrifuged and the supernatant dried under a nitrogen stream. After reconstituting in isogel-tris buffer, aliquots were assayed for PGD\textsubscript{2}, PGF\textsubscript{2a} and 6-keto-PGF\textsubscript{1a}. The radioimmunoassays used and the specificities of the antibodies have been described elsewhere.\textsuperscript{24, 25} Indomethacin (Merck, Sharp and Dohme, Rahway, NJ) was dissolved in dimethyl sulfoxide (DMSO, Sigma Chemical Company) at a concentration of 20 mg/ml. It was administered to each animal as 0.05 ml intraperitoneal injections (0.5 mg/injection) at 30 minutes before the operation and at every hour after occlusion. Thus, animals with carotid occlusion for 6 hours received a total of 6 injections containing 3.0 mg of indomethacin.

Brain edema was quantitated using the specific gravity technique of Nelson et al.\textsuperscript{26} Gerbils were sacrificed by cervical dislocation 6 hours after right CCA occlusion and the specific gravity of 4 to 6 samples of cerebral cortex from each hemisphere was measured.

The data were evaluated using Student's t-test or an analysis of variance.

Results

The right CCA was occluded in a total of 165 animals; of these 61 (37 percent) exhibited behavioral signs of unilateral cerebral ischemia and were regarded as "symptomatic." Approximately one-half of the symptomatic animals suffered episodes of running-jumping fits. These episodes occurred, usually, after one hour of occlusion. Eleven of these animals died before they were to be sacrificed and were not used in the study. In all cases death occurred during hypertonus, apparently as a result of respiratory arrest.

The effect of right CCA occlusion on the levels of PGD\textsubscript{2}, PGF\textsubscript{2a} and 6-keto-PGF\textsubscript{1a} in gerbil brain is shown in figure 1. PG levels increased in both hemispheres of the symptomatic animals. In general, the largest increase was seen in the left, non-occluded hemispheres. The levels of PGD\textsubscript{2} and PGF\textsubscript{2a} were elevated at 15 minutes and 2 hours after occlusion. PGF\textsubscript{2a} levels remained elevated at 6 hours but PGD\textsubscript{2} levels, while still slightly elevated, were not significantly greater than controls at 6 hours. The levels of 6-keto-PGF\textsubscript{1a} increased only slightly, becoming significantly greater only at 15 minutes of occlusion in the left hemispheres. The levels of A.A. metabolites in those animals which had running fits during occlusion were not significantly different from the levels measured in symptomatic animals which did not have these episodes. There was no change in the levels of A.A. metabolites in asymptomatic animals.

Indomethacin treatment inhibited the increase of PG levels in symptomatic animals (table). At both 2 and 6 hours, the levels of PGD\textsubscript{2} and PGF\textsubscript{2a} were found to be significantly lower in indomethacin treated animals than in animals receiving the vehicle, dimethyl sulfoxide (DMSO), alone. There was no difference in the levels of 6-keto-PGF\textsubscript{1a} in DMSO-treated symptomatics as compared to indomethacin-treated symptomatics.

Right CCA occlusion for 6 hours resulted in a decrease in the specific gravity of cortical samples taken from the right hemisphere of symptomatic animals (fig. 2). No change was observed in contralateral, non-occluded hemispheres or in asymptomatic animals. The specific gravity of cortical samples from animals treated with DMSO or with indomethacin dissolved in DMSO was greater than that of untreated animals. This is probably due to the
hygroscopic property of DMSO, since samples from both ischemic and nonischemic hemispheres of animals receiving DMSO were found to have greater values of specific gravity than samples from animals which were not injected. Indomethacin treatment had no effect on the development of brain edema since there was no difference in specific gravity of cortical samples from DMSO and indomethacin treated animals. Neither DMSO nor indomethacin treatment had any discernable effect on the proportion of animals with symptoms, or on the severity of symptoms after occlusion.

Discussion

 Interruption of the cerebral circulation by bilateral common carotid artery occlusion causes only small changes in the levels of A.A. metabolites in gerbil brain. However, unilateral CCA occlusion results in a marked increase in the levels of PGD<sub>2</sub> and PGF<sub>20</sub>. The difference is probably related to the amount of residual cerebral blood flow remaining after occlusion. With unilateral occlusion, flow is reduced enough to impair neuronal function in approximately 40 percent of animals, but there is still residual flow in most of these animals. This residual flow probably provides enough oxygen for the cyclooxygenation of arachidonic acid, resulting, after the subsequent reactions of the A.A. cascade, in the synthesis of the primary prostaglandins. In contrast, bilateral CCA occlusion causes total or near total reduction of cerebral hemispheric blood flow in most animals. In this situation the availability of oxygen would probably limit A.A. metabolism at the cyclooxygenation step.

In a few animals there may be a blood flow deficit in the contralateral, non-occluded hemisphere after uni-

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time of occlusion (hours)</th>
<th>N</th>
<th>PGF&lt;sub&gt;2&lt;/sub&gt; (ng/gm)</th>
<th>PGD&lt;sub&gt;2&lt;/sub&gt; (ng/gm)</th>
<th>6-Keto-PGF&lt;sub&gt;20&lt;/sub&gt; (ng/gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Right</td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td>Sham</td>
<td>7</td>
<td>0.5 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>1.0 ± 0.2</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>Vehicle</td>
<td>2</td>
<td>1.9 ± 0.4</td>
<td>7.2 ± 1.5</td>
<td>2.9 ± 0.6</td>
<td>15.2 ± 5.4</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>2</td>
<td>0.6 ± 0.2*</td>
<td>0.4 ± 0.2*</td>
<td>0.7 ± 0.1*</td>
<td>1.3 ± 0.2*</td>
</tr>
<tr>
<td>Vehicle</td>
<td>6</td>
<td>3.5 ± 0.8</td>
<td>8.5 ± 4.1</td>
<td>2.6 ± 0.8</td>
<td>4.0 ± 1.5</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>6</td>
<td>0.4 ± 0.1*</td>
<td>0.5 ± 0.1*</td>
<td>1.0 ± 0.3</td>
<td>1.0 ± 0.4</td>
</tr>
</tbody>
</table>

Intraperitoneal injections (0.06 ml) of vehicle (DMSO) or indomethacin (10 mg/ml) dissolved in DMSO were administered 30 minutes before, and every hour after occlusion. Values are mean ± standard error.

Significant difference from DMSO treated groups occluded for the same time intervals is indicated by * (p < 0.05) or by t (p < 0.1).
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1.0400 r
1.0420
1.0440 >>
1.0460 o
1.0480 ^

1.0500
1.0520
1.0540 Rr Ll

Rt Lt
Untreated
DMSO
Indomethacin
Rt Lt
Untreated
DMSO
Indomethacin
Rt Lt
Untreated
DMSO
Indomethacin

FIGURE 2. Effect of indomethacin on specific gravity changes in the cerebral cortex of gerbils subjected to unilateral CCA occlusion for 6 hours. Values are mean ± standard deviation of groups of 5 to 7 animals. The closed circle represents values obtained from both hemispheres of untreated, sham-operated animals. Indomethacin was dissolved in DMSO and administered as described in the text. Asterisks indicate a significant difference (p < 0.05, Student's t-test) from the ipsilateral hemispheres of untreated animals.

lateral CCA occlusion. Microscopic examination of the brain of animals with unilateral-occlusion have shown that ischemic damage is, in most instances, limited to the ipsilateral hemisphere. Damage to the contralateral hemisphere is rarely observed. However, despite a relative absence of histological damage in the contralateral hemisphere, bilateral alterations in brain chemistry and function are known to occur. There are bilateral changes in monoamine levels and energy metabolites in gerbil brains after unilateral occlusion. Bilateral alterations of cortical electrical activity have also been observed. In addition, Kogure et al. has found that unilateral cerebral embolization produces bilateral changes in cerebral energy metabolites and catecholamine levels in rat brain.

These observations may be related to changes in trans-callosal neuronal activity. Kempinsky found that unilateral lesions of the brain caused EEG depression in the contralateral, non-lesioned, hemispheres of cats. This was attributed to alterations in neuronal transmission across the corpus callosum since prior callosal sectioning prevented the contralateral EEG changes.

Diffusion of arachidonic acid, released from the ischemic hemisphere, to the non-ischemic hemisphere may explain increased PG levels in the contralateral hemispheres. The severe fits suffered by symptomatic gerbils also could contribute to elevated PG levels in both hemispheres since seizure activity is known to be accompanied by elevated PG levels in brain. However, it is doubtful whether these fits are responsi-
reflow") after brief interruption of the cerebral circulation. This would suggest that A.A. metabolites synthesized as a result of ischemia can impair the cerebral circulation.

Acknowledgment

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References

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R J Gaudet and L Levine

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