Postischemic Production of Eicosanoids in Gerbil Brain

O. Kempski, E. Shohami, D. von Lubitz, J.M. Hallenbeck, and G. Feuerstein

The postischemic production of PGE₂, PGD₂, 6-keto-PGF₁α, and TXB₂ in brain tissue was studied in Mongolian gerbils using tissue extraction as well as a new ex vivo method. This new method permits the study of prostaglandin synthesis in slices from discrete areas of the brain (cortex, hippocampus, striatum, hypothalamus). Gerbils were sacrificed at 0, 5, and 30 minutes, and 4 and 24 hours after a 15-minute occlusion of both carotid arteries. Apart from 6-keto-PGF₁α, tissue prostaglandins determined by the extraction method were significantly increased 3 and 30 minutes after reperfusion. These changes were most pronounced in the cortex, where PGE₂ and PGD₂ were increased 300% and 430%, respectively, 6 hours after reperfusion. At 24 hours after reperfusion, PGE₂ and PGD₂ synthesis were significantly decreased by 23-70% of the values obtained from sham-operated controls. Thromboxane increased slightly in all areas after recirculation and subsequently decreased to values below the control level in striatum. The results obtained by ex vivo incubation of tissue slices demonstrate that ischemia and subsequent recirculation cause site-, time-, and PG-specific changes of tissue eicosanoid production. (Stroke 1987;18:111–119)

DURING an ischemic cerebral insult, a multitude of physiological and biochemical parameters change rapidly and simultaneously. Up to now, the search for the most relevant pathophysiologic factor has not led to a conclusive answer. There is, however, increasing evidence that various parameters contribute to the secondary maturation of the brain insult, with delayed neuronal death and late blood-brain barrier (BBB) opening. Recent studies suggest that prostaglandins (PG's) might contribute to the physiologic consequences of brain ischemia through regulation of cerebral blood flow, vascular permeability, and modulation of excitatory or inhibitory transmitter effects.

In an attempt to evaluate the significance of PG's in cerebral ischemia, Gaudet and Levine showed that brain concentrations of PGD₂, PGE₂, PGF₂α, TXB₂, and 6-keto-PGF₁α were markedly elevated during the early recirculation phase in Mongolian gerbil brains. This observation was later confirmed by studies conducted on rats and by longer periods of ischemia in gerbils. However, several questions rose from these pioneering publications: Are tissue PG concentrations only temporarily increased because of arachidonic acid accumulation (the rate-limiting step of PG production) during ischemia; Is the removal of PG's produced during reperfusion impaired due to a disturbed microcirculation; Are the synthesis and/or degradation of PG's affected during reflow to favor accumulation of all the eicosanoids; Are different brain regions reacting more specifically than the crude homogenates from whole brain used in the cited publications?

To answer some of these questions, the capacity of discrete brain regions to produce PG's was examined by a new ex vivo technique. This method utilizes defined brain slices isolated from the brain at different times after ischemia, incubated under standard conditions in vitro, allowing the measurement of PG production during a given period. This approach reduces the influence of blood and plasma elements as well as accumulated arachidonic acid in the region since these elements are rinsed out by an initial wash prior to incubation of the slice. The data obtained by this method were compared to the PG content of the same brain regions using tissue extraction methods previously described. Furthermore, in contrast to previous studies that examined the effect of ischemia on brain PG's only during short periods (minutes or a few hours) after reperfusion, this study utilized an extended (24 hours) postischemic reflow protocol.

The results of the present study suggest that ischemia and reperfusion of brain tissue produce differential changes in eicosanoid production that are site-, time-, and PG-specific. In addition, this study also emphasizes the importance of subacute or chronic studies to obtain new insights on the neurochemical consequences of acute ischemic insults to the nervous system.

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Surgical Preparation

Mongolian gerbils, 12-15 weeks old, 50-70 g body wt (Tumblebrook Farms, N.Y.) were used in this study. The gerbils had access to food and water ad libitum until 3 hours prior to the experiment. The animals were anesthetized with 2% halothane in oxygen, and the body temperature was kept at 37° C with a heating blanket. Carotid arteries were exposed using microsurgical techniques, and ligatures were placed around both carotid arteries, which were simultaneously occluded by applying a defined tension (15 g) to the strings. Completeness of the occlusion was veri-

Brain Slices

Slices of brain tissue (1-2 mm³, 0.8-1 mm thick) were dissected from the parietal cortex, hippocampus, striatum, and hypothalamus and incubated in vitro using a previously described technique. In short, immediately after dissection, slices were washed with 1 ml of oxygenated (95% O₂, 5% CO₂) Krebs-Ringer solution containing NaHCO₃ (0.2%) and glucose (0.1%) and transferred to vials containing 1 ml of the same solution for 1-hour incubation in a shaking bath at 37° C.

At the end of the incubation period, the supernatant was aspirated and stored at -70° C for PG analysis. The tissue was homogenized in water, and proteins were measured according to Lowry et al.

Tissue Extract

To compare data obtained by the ex vivo method with extraction data reported previously, brain PG content was also analyzed after extraction from whole brain regions. Two approaches were chosen: In Group 1, the decapitated gerbil heads were immediately frozen by immersion in liquid nitrogen. The procedure took less than 4 seconds. In Group 2, decapitation was followed by fast brain removal (< 40 seconds) and freezing on dry ice. Brain tissue was stored at -70° C until assayed. Single hemispheres were homogenized in Tris-EDTA buffer (0.05 M Tris-NaCl:0.2 M EDTA 9:1, pH 7.0) using a Polytron homogenizer (Brinkmann Instruments, Westbury, N.Y.). Homogenates were centrifuged and the supernatants washed 3 times with ether (96-98%, Mallinckrodt), and the aqueous phase was used for the radioimmunoassay (RIA).

Radioimmunoassay

For the initial experiments, PGE₂, 6-keto-PGF₁α, and TXB₂ were determined using RIA kits purchased from New England Nuclear (Boston, Mass.). The radioactive labels were iodine-125 for PGE₂ and tritium for 6-keto-PGF₁α and TXB₂. The antibodies had < 2.5% cross-reactivity with all other PG's. Subsequently, tritiated ligands were purchased from New England Nuclear (100-200 Ci/mmole), and antisera against PGE₂, PGD₂, TXB₂, and 6-keto-PGF₁α were obtained from L. Levine (Brandeis University, Waltham, Mass.). Anti-PGE₂ has a 100% cross-reactivity with PGE₁, PGA₂, and PGE₂. Anti-PGD₂ cross-reacts at 8% with PGE₂ and at < 1% with TXB₂ and 6-keto-PGF₁α. Cross-reactivities for anti-6-keto-PGF₁α were also < 1% with PGE₂, PGD₂, and TXB₂. Detailed specifications of these antibodies were previously described.

Unlabelled PG standards were purchased from Sigma Chemical Co (St. Louis, Mo.). The RIA was carried out in 0.1 M potassium phosphate buffer, pH 7.4, containing 0.1% sodium azide and 0.1% bovine serum albumin as previously described. Samples or standards were incubated with antiserum and radioligands for 18-24 hours at 4° C. Bound and free fractions were separated with dextran-coated charcoal (Norit GSX Activated, Sigma Chemical Co). Tritium was counted in a liquid scintillation counter (LKB 1218 Rackbeta) and iodine-125 by a gamma counter (LKB 1270 Rack-gamma II).

Statistical Analysis

Data in text and figures are given as means ± SEM for the indicated number of experiments. Analysis of variance (ANOVA) followed by the Student-Newman-Keuls test for multiple comparisons was used to evaluate group differences. Student's t test was used to evaluate percent changes from controls. Data were considered different with an α error probability of < 5% (two-tailed tests).

Results

Tissue Extraction

The concentrations of PG's extracted from brain homogenates after 15 minutes of ischemia and 5 or 30 minutes of recirculation are summarized in Figure 1. Each time point shows 2 columns, representing data from brains frozen instantly in liquid nitrogen while still in the skull (Group 1) or placed on dry ice after being freed from surrounding tissues (Group 2, “Materials and Methods”). Two control groups, in which 5 or 30 minutes of “wake-up time” was allowed after sham operation, are also included. These 2 control groups did not differ significantly. However, there was a substantial difference between the two freezing methods. TXB₂ was not detectable in liquid-nitrogen-frozen brains, whereas brains frozen on dry ice showed high levels (p < 0.05). Similar differences were found immediately after ischemia. PGE₂ levels in Groups 1 and 2 followed the trend described for TXB₂, but these changes did not reach statistical significance.
levels were not significantly changed by rapid freezing in liquid nitrogen.

PG levels remained unchanged after 15 minutes of ischemia. After 5 minutes of recirculation, however, significant increments of PGD₂, TXB₂, and PGE₂ were found; these PG's were still elevated 30 minutes after the onset of reperfusion (Figure 1).

**Ex Vivo PG Production**

Brain slices from cortex, hippocampus, striatum, and hypothalamus produced easily assayable amounts of all PG's studied. In the 1-hour incubation, up to 30-fold more PG's were released into the medium than were extracted from the tissue (Table 1). These data demonstrate the sustained capacity of brain tissue to synthesize PG's in vitro. The predominant PG class was PGD₂, which exceeded the other PG's by up to an order of magnitude. Regional variability of the basal PG production in sham-operated animals is listed in Table 2.

The most pronounced changes during the reperfusion period were found for PGE₂ and PGD₂, with increases of up to 300% (Figures 2 and 3). Many changes observed were time-specific or reached significance only in defined brain areas. Data from the hypothalamus did not always reflect changes in the other areas, probably because bilateral carotid occlusion in gerbils does not produce complete ischemia in the hypothalamus. Thus, some oxygen could be supplied to this area during the occlusion.

Cortex, hippocampus, and striatum showed a trend toward increased PGE₂ production during the first 4 hours of recirculation as confirmed by regression analysis (Figure 2). In the hippocampus and striatum this tendency reached significance in the ANOVA (p < 0.025 and p < 0.001, respectively). After 24 hours, PGE₂ production decreased significantly (p < 0.001) in all the brain regions studied.

In contrast to PGE₂, PGD₂ was produced at the highest rates immediately after reperfusion. Changes in
Table 1. Basal Levels of Prostaglandins Extracted or Released From the Brain Tissue of Sham-Operated Gerbils Sacrificed 5 Minutes After Termination of the Halothane Anesthesia

<table>
<thead>
<tr>
<th>Prostaglandin</th>
<th>Extraction* (pg/mg protein)</th>
<th>Release† (pg/mg protein/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGD₂</td>
<td>134 ± 37</td>
<td>3200 ± 225</td>
</tr>
<tr>
<td>PGE₂</td>
<td>175 ± 50</td>
<td>1422 ± 249</td>
</tr>
<tr>
<td>TXB₂</td>
<td>25 ± 8</td>
<td>234 ± 24</td>
</tr>
<tr>
<td>6-keto-PGF₆a</td>
<td>25 ± 6</td>
<td>196 ± 36</td>
</tr>
</tbody>
</table>

*PG's were extracted from 1 hemisphere after decapitation and freezing the brain on dry ice (Group 2, "Materials and Methods"). 5-7 gerbils were used for this study.

†Cortical slices were incubated for 1 hour in oxygenated Krebs-Ringer solution (see "Materials and Methods"). 6-9 gerbils were used for this study.

PGD₂ were especially pronounced in the cortex and hypothalamus, where increases reached 200-300% of control levels (Figure 3). In the striatum, PGD₂ rose significantly after 5 minutes of reperfusion, but after 4 hours of reperfusion, PGD₂ production was reduced in all areas to less than 50% of the control level. Decreased levels of PGD₂ were still present after 24 hours.

The early increases of TXB₂ (Figure 4) were far less evident than those of PGD₂. Nevertheless, there was a tendency toward higher levels after 5 minutes of reper-

Table 2. Prostaglandins Released Into Incubating Medium in 1 Hour by Brain Slices of Sham-Operated Gerbils

<table>
<thead>
<tr>
<th>Prostaglandin</th>
<th>Cortex</th>
<th>Hippocampus</th>
<th>Striatum</th>
<th>Hypothalamus</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGD₂</td>
<td>Mean</td>
<td>3.2</td>
<td>2.9</td>
<td>2.4</td>
</tr>
<tr>
<td>SEM</td>
<td>0.2</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>n</td>
<td>35</td>
<td>19</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>PGE₂</td>
<td>Mean</td>
<td>1.422</td>
<td>2.144</td>
<td>0.218</td>
</tr>
<tr>
<td>SEM</td>
<td>0.249</td>
<td>0.308</td>
<td>0.026</td>
<td>0.177</td>
</tr>
<tr>
<td>n</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>TXB₂</td>
<td>Mean</td>
<td>0.234</td>
<td>0.283</td>
<td>0.226</td>
</tr>
<tr>
<td>SEM</td>
<td>0.024</td>
<td>0.041</td>
<td>0.026</td>
<td>0.189</td>
</tr>
<tr>
<td>n</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>6-keto-PGF₆a</td>
<td>Mean</td>
<td>0.196</td>
<td>0.149</td>
<td>0.041</td>
</tr>
<tr>
<td>SEM</td>
<td>0.036</td>
<td>0.035</td>
<td>0.015</td>
<td>0.017</td>
</tr>
<tr>
<td>n</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
</tr>
</tbody>
</table>

n = number of slices. Mean values from sham-operated animals sacrificed at varying times after halothane exposure are given since there were no significant differences between the groups of sham-operated animals.

Figure 2. Production of PGE₂ by tissue slices. Slices were taken from the cortex, hippocampus, striatum, and hypothalamus of gerbils at various times of recirculation (0, 5, and 30 minutes, 4 and 24 hours) after a 15-minute bilateral carotid occlusion. PG production is expressed in percent of values obtained from sham-operated controls after identical wake-up times from anesthesia. Mean ± SEM calculated from 6-9 experimental animals and the same number of controls in each group. Groups were compared by ANOVA and Student-Newman-Keuls test and are considered different with an α error probability of 5%. *p < 0.05, **p < 0.01 vs. sham-operated controls; †significant vs. 4-hour group at p < 0.01.
Figure 3. Production of PGD\(_2\) by tissue slices. Details as in Figure 2.

Figure 4. Production of TXB\(_2\) by tissue slices. Details as in Figure 2, except *p < 0.05, **p < 0.01, †p < 0.001 vs. sham-operated controls.
fusion which, however, reached significance only in hypothalamus ($p < 0.01$). Later (4 hours after reperfusion), TXB$_2$ production in the striatum decreased and reached a minimum of 44% of control ($p < 0.001$). In all 4 brain areas studied except the striatum, TXB$_2$ production returned to control levels 24 hours after the onset of reperfusion, in marked contrast to PGE$_2$ and PGD$_2$.

Alterations of 6-keto-PGF$_{1α}$ (Figure 5) were region-specific; no time-dependent trends were apparent. There was doubling of 6-keto-PGF$_{1α}$ production in the hippocampus after 30 minutes of recirculation and a subsequent decline to 60% of control values after 24 hours ($p < 0.05$ vs. 30-minute group). In the hypothalamus, 6-keto-PGF$_{1α}$ production was increased immediately after ischemia and dropped significantly within 5 minutes of recirculation ($p < 0.01$). Twenty-four hours after the ischemic insult, 6-keto-PGF$_{1α}$ production was not significantly different from the control levels across the brain regions studied.

**Discussion**

The diversity of the PG cascade and the short half-lives of most of its intermediate products preclude an undisturbed study of the significance of eicosanoids in cerebral ischemia and reperfusion. Although numerous reports on the biological effects of PG’s have been published in recent years, the functions of PG’s in the central nervous system (CNS) are still far from being understood.$^{16,17}$ Therefore, a discussion of PG actions in the CNS must be based on 3 considerations:

1. Individual PG’s have a variety of sites of action in the brain, and they act on many cell types. PG’s may modify the BBB via endothelial, smooth muscle, or glial cells$^{16,19}$; interactions of PG’s with thrombocytes may change their adhesiveness and aggregability and the release of vasoactive substances. Very little is known about PG effects on glial and nerve cells and thereby on brain function.

2. All these actions should be evaluated according to their quality. Are PG’s mediators of pathologic damage (e.g., BBB breakdown, platelet aggregation, disturbed neuronal function) or is PG release part of a tonic input that is important in normal function (e.g., patency of the microcirculation, modulation of neurotransmitter action)?

3. The release of PG’s is site-specific. Regional differences can be explained by different criteria. One reason likely to contribute to the PG pattern found for individual brain regions is the diversity of cell types which produce PG’s.$^{20-22}$ Depending on the cell type, the release of PG’s under pathophysiological conditions has a different significance.

These considerations taken together suggest that information on changes in brain PG concentration after ischemia is not enough to either identify pathophysiologically relevant factors or to interfere therapeutically. The *ex vivo* method in this study is an additional approach to study the conversion of arachidonate to PG’s in tissues, which represents the endogenous rate of arachidonate turnover. The advantages of the method are several-fold: 1) since the accumulated release of

![Graph](image-url)
the PG is studied, smaller tissue samples can be examined, allowing the comparison of more discrete brain regions; 2) the influence of blood cells is reduced since samples are free of large vessels and are carefully washed in incubation medium prior to the experiment; 3) no plasma factors other than those retained by the tissue block are interacting with the tissue during the incubation period; 4) tissue PG's, initially elevated due to release of arachidonic acid during ischemia, are also washed out prior to incubation. During ex vivo studies with normal brain slices, PG's in the incubation medium increase for up to 3 hours. This increase reflects PG synthesis and not just the diffusion into the medium of PG's present in the tissue at explantation. Moreover, the actual amounts of PG's (per milligram of tissue protein) found in the medium after 1 hour of ex vivo incubation were 8–25 times higher (depending on the specific PG) than those extracted from the tissue (Figure 1; Table 2). In addition, an increased ex vivo production of eicosanoids during reperfusion does not just reflect changes in tissue concentrations of PG precursors. This is demonstrated by the increased ex vivo production of 6-keto-PGF₁₀ and PGE₂ after 30 minutes and 4 hours of reperfusion respectively, and the decreased synthesis of PGE₂ after 24 hours and of PGD₂ after 4 and 24 hours. At these times brain levels of arachidonic acid are back to normal in gerbils. The evolving pattern of eicosanoid production (early increase of PGD₂, later increase of PGE₂) indicate changing activities of rate-limiting enzymes. Thus, the ex vivo method, unlike the extraction techniques so commonly used, measures de novo PG synthesis and release after the primary ischemic insult. However, to compare the ex vivo data with findings reported by others and especially to validate data obtained by the new method, it was also important to analyze tissue PG concentrations. Two approaches were chosen: rapid freezing in liquid nitrogen after decapitation, and freezing of the explanted brains on dry ice. The latter method better reflects PG levels expected in brain slices from ex vivo experiments, in which brains were removed in the same way.

The extraction data confirm the results of Gaudet and Levine and Gaudet et al who found steep increases of PGD₂, TXB₂, and PGE₂ 5 minutes after recirculation. The differences between the two freezing methods show that the brief ischemic period caused by removing the brains and freezing them on dry ice was enough to cause significant alterations of TXB₂. Actual PG levels are best conserved by microwave heating of the head, which instantly denatures all enzymes necessary for PG synthesis or degradation. Freezing in liquid nitrogen, however, also produces reliable results as seen by the undetectable levels of TXB₂ in control brains under that condition. Relative changes in PG levels during recirculation are identical when measured with both freezing methods.

PGD₂ levels did not increase in the brains of sham-operated controls that had been frozen on dry ice (Figure 1) although changes during recirculation were pronounced. This suggests that the production of PGD₂ is regulated by mechanisms other than those controlling the formation of TXB₂ and PGE₂. The production of PGD₂ may be more closely controlled because of its proposed function as a neuromodulator. Furthermore, PGD₂ synthesis (studied in brain microvessels) has a pH optimum of 8.0. At pH 6.5, a value which can be reached in ischemia, PGD₂ formation is reduced to 10% of its normal level. If this observation is extrapolated for brain parenchyma, it may explain the relative resistance of PGD₂ formation to brief episodes of ischemia. The ex vivo results were not affected by this pH dependency since slices were incubated under near-optimal pH conditions. Therefore, the immediate increase in PGD₂ formation in the slices after ischemia is probably due to high levels of arachidonic acid accumulating in the slice during ischemia and the abundance of oxygen in the medium.

If PGD₂ synthesis is inhibited in ischemic tissue, available arachidonic acid might be transformed into other eicosanoids. The immediate rise of TXB₂ and, to a lesser degree, PGE₂ extracted from the brains of sham-operated controls frozen on dry ice (Figure 1) probably reflects the sudden increase of arachidonic acid during the first minute after decapitation. TXB₂ may originate from platelets and glial cells, while PGE₂ is probably released from vascular elements and glial cells.

Analysis of the ex vivo data reveals that this approach allows the study of postischemic PG metabolism from a new point of view. In contrast to tissue extraction, the increase in PG synthesis during the early reperfusion period was significant only for PGD₂. PGE₂, on the other hand, showed a tendency to increase during the first 4 hours after reperfusion. One day after the stroke, PGE₂ and PGD₂ were produced at reduced rates.

To evaluate these changes in detail, it might be useful to distinguish effects that are expressions of an ongoing pathological process from those reflecting re-action of the brain to counteract such damaging events. Self-protective mechanisms may act on the microcirculation as well as on brain metabolism. Protective functions have been attributed to PGI₂ and PGD₂. PGI₂ is a potent vasodilator and reduces platelet aggregation through a cAMP-mediated mechanism. PGI₂ (in combination with indomethacin and heparin) prevents postischemic reperfusion impairment in dogs after global ischemia. PGI₁ administration after middle cerebral artery (MCA) occlusion in cats reduced the extravasation of protein-bound dyes such as Evans blue, which signals preservation of the BBB.

Changes in 6-keto-PGF₁₀, the stable metabolite of PGI₁, in the present study were pronounced only in the hippocampus. Since the hippocampus is most sensitive to ischemia the enhanced production of PGI₁ could be an attempt of the injured tissue to improve the local microcirculation.

PGD₂, on the other hand, has been proposed as a modulator of excitatory and inhibitory transmitter actions, potentiating especially the effects of GABA. In addition, PGD₂ was shown to hyperpolarize the plasma...
membrane of Purkinje cells in culture. Transmitter release from brain synaptosomes is apparently not affected by PGD2. PGD2 has been shown to have hypnotic actions, and it has been postulated that reduced production of PGD2 is responsible for spontaneous convulsions in a strain of gerbils. Since enhanced glutamate release has been suggested as a possible mediator of ischemic brain damage, a modulatory role of PGD2, i.e., potentiating inhibitory inputs, might be seen as an endogenous defense mechanism.

PGD2 production decreased in all brain areas tested 4 or 24 hours after reperfusion. This phenomenon has not been described before. If this PG should have a protective function in the brain, the defense mechanism may be disrupted at that time and therefore contribute to the late neuronal death seen after ischemia. Interestingly, PGD2 release is lowest in the hippocampus, which is most susceptible to neuronal hyperexcitation and damage after ischemia. More prolonged time points after reperfusion will be necessary to further clarify this issue.

The gradually increasing output of PGE2 is not easy to explain. PGE2 is a vasoactive prostanoid that can cause vasoconstriction or vasodilation dependent on species and blood vessel type (for review see Reference 40). E-type PG's can cause edema and signs of inflammation or potentiate edema and inflammatory reactions produced by other agents (e.g., leukotrienes; for review see Reference 42). These observations indicate that excessive PGE2 production might be an unwanted phenomenon after ischemia. On the other hand, PGE2 — like PGD2 — causes sedation when injected into the cerebral ventricles and has been described as a modulator of neuronal function (for review see Reference 41). PGE2 inhibits stimulation-induced norepinephrine release from rat cortical slices. Interestingly, PGE2 levels were significantly decreased after one day of reperfusion in a fashion similar to PGD2. Further studies using injection of PGE2 or PGD2 antagonists may yield further indications of the role of E-series PG's in ischemia.

Very little is known about the effects of thromboxane on neuronal functions, but it has been shown that apart from platelets, glial cells are apparently capable of producing thromboxanes. Once formed, TXA2 causes vasoconstriction and platelet aggregation. Several reports by Hallenbeck et al.10,31,46 suggest that platelet activation, presumably triggered by thromboxane, participates in the pathogenesis of the impaired microcirculation produced by global and focal ischemia. A combination therapy of heparin, indomethacin, and PG12 reduced the ischemic damage.11 These studies suggested detrimental effects of thromboxane very early after the release of compression-induced ischemia. The early increase of TXB2 in the present study points in the same direction. At later times of reperfusion, TXB2 formation was reduced. A therapeutic intervention to restrict the production or effects of this important prostanoid early after the insult should be considered.

The results of this study confirm the changes in eicosanoid production obtained by tissue extraction and point out the significance of changing pathways of prostanoid production during recirculation. The late increase in PGE2 production as well as the dramatic increase in PGD2 formation make these two PG species interesting candidates for further studies. The significant suppression of PGD2 and PGE2 late after ischemia might be related to the delayed neuronal death seen in this model of global ischemia. Future research should focus on the site-specific action of PG's and identify cell types responsible for their release.

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References


10. Irvine RF: How is the level of free arachidonic acid controlled in mammalian cells? J Biochem 1982;204:3-16


27. Kirino T: Delayed neuronal death in the gerbil hippocampus following ischemia. *Brain Res* 1982;239:57–69
35. Seregi A, Forstmann U, Hertting G: Decreased levels of brain cyclooxygenase products as a possible cause of increased seizure susceptibility in convulsion-prone gerbils. *Brain Res* 1984;305:393–395
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