Prostaglandin Profiles in Relation to Local Circulatory Changes Following Focal Cerebral Ischemia in Cats

Tetsu Hanamura, MD, Taku Shigeno, MD, DMSc, Takao Asano, MD, DMSc, Tatsuo Mima, MD, and Kintomo Takakura, MD, DMSc

We explored the temporal and topographic relations between local cerebral blood flow and regional brain prostaglandin profile following prolonged or transient occlusion of the middle cerebral artery in cats. Each experimental group was subjected to a sham operation, prolonged ischemia, or recirculation. Local cerebral blood flow was measured by the hydrogen clearance method. Following in situ freezing, cortical samples were obtained from each gyrus for determination of prostaglandin (PG) F$_{2\alpha}$, PGE$_2$, 6-keto-PGF$_{1\alpha}$, and thromboxane (TX) B$_2$ concentrations by radioimmunoassay. During prolonged ischemia, the concentrations of PGF$_{2\alpha}$ and PGE$_2$ within the middle cerebral artery territory were significantly increased. Immediately after recirculation, there was a prominent but transient increase in PGF$_{2\alpha}$ and PGE$_2$ in gyri that had been exposed to moderate ischemia (perifocal area). By contrast, the increases in these prostaglandins were slow and less prominent in gyri that had been exposed to severe ischemia (the focal area). The concentration of 6-keto-PGF$_{1\alpha}$ did not change during prolonged ischemia but transiently increased following recirculation in both the focal and perifocal areas. The TXB$_2$ concentration did not change in any experimental group. Our study revealed a homogeneous increase in the regional brain content of PGE$_2$ or PGF$_{2\alpha}$ in spite of the heterogeneous reduction of local cerebral blood flow during prolonged ischemia. Following recirculation, the focal and perifocal areas exhibited different patterns of prostanoid content. No correlation was found between local cerebral blood flow and the regional concentration of any prostaglandin examined. (Stroke 1989;20:803–808)

A rachidonic acid comprises the major portion of free fatty acids, which are liberated from membrane phospholipids following cerebral ischemia. A small portion of liberated arachidonic acid is then incorporated into the arachidonic cascade, namely the cyclooxygenase and lipoxygenase pathways, which are converted into a variety of eicosanoids. Because of their potent biologic activities, these eicosanoids may be involved in the regulation of local cerebral blood flow (ICBF) or possibly local tissue injury and edema formation. In this regard, most previous studies have been carried out using models of continuous or transient global ischemia, and only a few reports have surfaced dealing with prostaglandin (PG) synthesis following regional cerebral ischemia. In particular, the relation between the alteration of ICBF and the topographically corresponding changes in PG synthesis following regional ischemia in large animals has not yet been surveyed.

Our present study was thus undertaken to analyze the topographic as well as the temporal relations between ICBF and the brain PG profile (PGF$_{2\alpha}$, PGE$_2$, 6-keto-PGF$_{1\alpha}$, and thromboxane [TX] B$_2$) following occlusion of the middle cerebral artery (MCA) in cats, which may be important in understanding the heterogeneous character of the pathophysiologic events following regional cerebral ischemia.

Materials and Methods

We anesthetized 31 adult cats of either sex weighing 2.5–4 kg with 1% halothane, and a tracheostomy was performed. The femoral artery and vein were catheterized for monitoring systemic arterial blood pressure, for periodic arterial blood gas analysis, and for the administration of fluids and drugs. A fine catheter filled with heparinized saline was introduced into the left lingual artery for later brain
perfusion. The cat's head was then fixed to a stereotactic operation frame. The left MCA was exposed by the transorbital approach using an operating microscope. For the measurement of ICBF, platinum electrodes were inserted into the cerebral cortex at six locations over the parasylvian area (the posterior sylvian, middle ectosylvian, and posterior ectosylvian gyri) through small burr holes. Thus, at least one electrode was placed in each gyrus. During these procedures, special care was taken to prevent even minimal mechanical or thermal damage to the cerebral cortex and to prevent the spread of blood into the subarachnoid space.

After the above procedures, halothane was discontinued. The cats were immobilized by gallamine and ventilated mechanically with a 75% N₂O:25% O₂ mixture. The systemic arterial blood pressure, body temperature, end-tidal Pco₂ and P₀₂ were continuously monitored. Arterial blood gases were checked at regular intervals. These parameters were maintained within physiologic ranges throughout the experiment. The left MCA was subsequently occluded by applying a clip at its origin from the internal carotid artery.

Regional cerebral blood flow in each parasylvian gyrus was measured by the hydrogen clearance method using platinum electrodes inserted into the cerebral cortex. The electrodes were made of 25-μm platinum-iridium wires insulated except for the terminal 0.5 mm. Control ICBF was measured at least twice before MCA occlusion. In the prolonged ischemia groups, ICBF was measured 10 minutes after MCA occlusion and every 30 minutes thereafter. In the recirculation groups, the first measurement of posts ischemic ICBF was started immediately after removal of the clip and thereafter was repeated every 30 minutes.

At least one (as many as three) electrodes were inserted into each gyrus. The ICBF value obtained with one electrode represents that of a tissue bulk of the brain. Since a much larger amount of brain tissue was needed for determining PG content, it was not possible for us to obtain a strictly topographic correlation between ICBF and brain PG content. However, since ICBF of adjacent electrodes within a gyrus exhibited minimal differences, the mean ICBF of these electrodes may be considered to represent the mean ICBF of the gray matter in that gyrus. On these grounds, we analyzed the topographic correlation between ICBF and brain PG content.

The cats were divided into the following experimental groups: sham-operated (n=4), 2-hour prolonged ischemia (n=5), 4-hour prolonged ischemia (n=7), 2 hours of ischemia followed by 5 minutes of recirculation (n=5), 2 hours of ischemia followed by 30 minutes of recirculation (n=5), and 2 hours of ischemia followed by 2 hours of recirculation (n=5).

At the end of the experiment, the calvaria was widely exposed down to the lateral margin of the skull base, and a plastic funnel was placed to surround the skull. The catheter in the lingual artery was then advanced into the common carotid artery, and brain perfusion with ice-cold saline was started at a pressure exceeding the systemic arterial blood pressure. Simultaneous with the start of brain perfusion, the clip on the MCA was removed and the left external jugular vein was cut and bled freely to facilitate the exchange of intracerebral blood with the perfusate. Several seconds later, liquid nitrogen was poured into the funnel and the brain was frozen in situ according to the method of Welsh et al. 12

After the in situ freezing (which was continued for 15 minutes), the cat was killed with the intravenous injection of a saturated KCl solution. During the continuous pouring of liquid nitrogen, each gyrus was resected using an electric drill. In the same way, the white matter and surface vessels of each sample were removed as much as possible. Samples obtained from each gyrus were separately compiled and stored at -80°C for later determination of PG content.

Brain samples were freeze-dried for 10 days, and the dry weight was measured. Each sample was then homogenized in ice-cold chloroform, extracted with Folch's solution (methanol:chloroform 1:2), and dried under a nitrogen stream (N₂). The residue was dissolved in lower-phase (LP) solution formed by the mixture of chloroform:methanol:HCl-water (pH 2) 200:100:75, applied to a Sephadex G-25 column, eluted with LP solution, and dried under N₂. To remove the nonesterified fatty acids, carbon tetrachloride and 10% methanol in a 0.1 M phosphate buffer solution were added to the residue, which was then centrifuged at 3,000 rpm for 10 minutes. A buffer layer was obtained, adjusted to pH 3.0 by adding 3N HCl, and further centrifuged at 3,000 rpm for 10 minutes together with ethyl acetate. The buffer layer was again obtained, added to methanol and 0.1N NH₄OH, and dried under N₂. The residue was dissolved in methanol and applied to high-performance thin-layer chromatography plates for the separation of PGs. Each residue sample was divided into two parts, one for radioimmunoassay and the other for measurement of PG recovery rate. The concentration of each PG was obtained from individual PG standard curves and corrected according to the recovery rate of each sample.

Our results are expressed as mean±standard deviation. The data was analyzed by both the Kruskal-Wallis and the Mann-Whitney methods. Differences were considered significant only when both methods yielded significant differences (p<0.05).

Results

Systemic arterial blood pressure remained stable during the experiment without any significant differences among groups. PaCO₂ and PaO₂ were frequently checked and by adjusting the respiratory rate remained between 30 and 35 and 100 and 130
mm Hg, respectively. At the start of the experiment, the concentrations of serum sodium, potassium, and chloride were 152±2, 3.5±0.5, and 118±3 meq/l, respectively. Serum osmolarity was 315±5 mosm/l. These parameters fluctuated little throughout the experiment. Rectal temperature was kept between 37.5° and 38.5° C by the use of a warming blanket and an overhead infrared lamp.

Since we examined the relations between ICBF and the PG profile within the gray matter, the ICBF data of the electrodes, the control values of which were <60 ml/100 g/min, were excluded from data analysis because of possible mechanical or thermal damage to the cerebral cortex. Control ICBF in all groups was 96±16 ml/100 g/min. In the prolonged ischemia groups, the ICBF of each electrode remained fairly stable following MCA occlusion, although ICBF during the late ischemic phase tended to be slightly lower than that during the early ischemic phase. As expected, in most cats the ICBF of the electrodes located within a gyrus showed parallel changes. In the recirculation groups, an immediate recovery of ICBF (up to 89±25 ml/100 g/min) was observed; ICBF tended to decline (although not significantly) thereafter, representing the occurrence of posts ischemic hypoperfusion (Figure 1).

In the sham-operated group, the control tissue concentrations of PGF$\text{_{2a}}$, PGE$\text{_{2}}$, TXB$_{2}$, and 6-keto-PGF$_{1a}$ were 39.9±31.5, 48.7±37.2, 127.0±120.7, and 159.0±136.2 pg/mg dry wt, respectively. The relations between mean CBF during MCA occlusion (ischemic ICBF) and the tissue concentration of each PG in the continuous ischemia groups are shown in Figure 2. In these groups, the tissue concentrations of PGF$\text{_{2a}}$ and PGE$\text{_{2}}$ were significantly greater than control ($p<0.01$). Concentrations of PGF$\text{_{2a}}$ and PGE$\text{_{2}}$ in the 2-hour ischemia group were 428.9±275.0 and 246.0±108.1 pg/mg dry wt, respectively. In the 4-hour ischemia group, the concentrations of PGF$\text{_{2a}}$ and PGE$\text{_{2}}$ were 251.6±269.3 and 170.7±125.7 pg/mg dry wt, respectively. Nevertheless, the increase in each PG was not significant. No significant linear correlation was found between ischemic ICBF and tissue concentration of PGF$_{2a}$ or PGE$_{2}$.

The tissue concentration of 6-keto-PGF$_{1a}$ remained within the control range in the 2-hour ischemia group (mean±SD 108.9±65.1 pg/mg dry wt). Although it was slightly elevated in the 4-hour ischemia group (mean±SD 246.5±179.9 pg/mg dry wt), the increase was not significant. No linear correlation was found between ischemic ICBF and brain concentration of 6-keto-PGF$_{1a}$. The TXB$_{2}$ concentration showed no significant change.

The relation between ischemic ICBF and brain concentration of each PG in the recirculation groups is shown in Figure 3. The brain concentrations of PGF$_{2a}$ and PGE$_{2}$ were markedly elevated (3,000 pg/mg dry wt) 5 and 30 minutes after recirculation. This tremendous increase in each PG occurred only in those gyri that had been moderately ischemic (ischemic ICBF between 15 and 25 ml/100 g/min).
during MCA occlusion, and it rapidly subsided in 2 hours. On the other hand, the severely ischemic gyrus (ischemic ICBF of < 15 ml/100 g/min) did not show an appreciable change in its PG content during the early recirculation period. A moderate increase in PGF$_2\alpha$ and PGE$_2$ concentrations was noted only 2 hours after recirculation.

A significant increase in 6-keto-PGF$_{1\alpha}$ concentration was found only in the 5 minutes of recirculation group (mean ± SD 573.3 ± 226.6 pg/mg dry wt, p < 0.01). The increase was more marked in the focal than in the perifocal area. However, no correlation was found between the ischemic (Figure 3) or postischemic (Figure 4) ICBF and brain concentrations of 6-keto-PGF$_{1\alpha}$ in the recirculation groups. The alteration of brain TXB$_2$ concentration follow-

### Discussion

In most previous studies, the alteration of brain PG concentration following cerebral ischemia has been surveyed by the use of global ischemia models in small animals. Through these studies, the alterations in brain PG concentrations following complete or incomplete global ischemia, permanent or transient, have been well delineated. However, there is a paucity of literature regarding regional brain PG concentrations following regional ischemia. Although regional changes in brain PG content following unilateral or bilateral common carotid artery occlusion in gerbils have been reported, it is well known that in symptomatic gerbils, this model causes severe ischemia in both hemispheres. Therefore, results obtained from this model represent those of incomplete global rather than regional ischemia. Clearly, regional ischemia models in larger animals would be more desirable to study the regional heterogeneity in brain PG synthesis related to ICBF and edema formation.

In this respect, the cat MCA occlusion model seems to be an appropriate one, except for the problem of brain sampling. Determination of brain levels is fraught with artifacts that are caused mainly by improper methods of sampling. Particularly in experiments using large animals, it is not possible to use conventional methods such as freezing after decapitation or microwave fixation. Thus, we employed the in situ freezing technique of Welsh et al., which causes minimal alterations in brain energy metabolites, especially of the gray matter, during the procedure. This technique was reinforced by simultaneous brain perfusion with ice-cold saline, which helped to instantaneously inactivate brain cyclooxygenase and to lessen the contamination of PGs formed in the blood.

Although the reported PG concentrations in normal brain differ widely depending on species and techniques, the control PG concentrations we obtained were in a range similar to or a little greater than those obtained in smaller animals. Regarding brain PG concentrations in cats, Ellis et al. have previously reported control values of PGE$_2$ (216 ± 44 ng/g wet wt) and PGF$_2\alpha$ (210 ± 48 ng/g wet wt) much higher than ours. This discrepancy may be ascribed to differences in the method of brain sampling.

Our study revealed that brain concentrations of PGE$_2$ and PGF$_2\alpha$ within the ischemic MCA territory were moderately increased. In addition, brain concentrations of these PGs had no linear correlation with the severity of ICBF reduction in the observed range (10–25 ml/100 g/min) during 2 hours of ischemia. This result is of interest because the ICBF threshold for cell membrane depolarization accompanied by intracellular Ca$^{2+}$ influx and subsequent phospholipase activation has been estimated to be
<10 ml/100 g/min. Thus, the observed increase in PG concentrations is not attributable to cell membrane depolarization due to energy depletion. As possible causes, the following may be considered: 1) phospholipase activation not through energy depletion, similar to activation of the phosphatidylinositol cycle through stimulation of membrane receptors by a variety of neurotransmitters and other agonists; 2) existence of minute ischemic foci within the area, which is suggested by the well-documented microregional heterogeneity of brain energy metabolism following regional ischemia; 3) diffusion of PGs into the extracellular space, which could be inferred from the fact that the brain has no enzymes to metabolize these PGs.

In our study, the brain concentrations of 6-keto-PGF\textsubscript{1α} and TXB\textsubscript{2} did not change following continuous MCA occlusion. This had been anticipated with TXA\textsubscript{2} because this PG, being mainly produced in platelets, would be washed out to a large extent by brain perfusion. The brain concentration of 6-keto-PGF\textsubscript{1α}, which is synthesized mainly in the vascular endothelium, could be influenced by brain perfusion to some extent, but probably less than that of TXA\textsubscript{2}. This is suggested by increased 6-keto-PGF\textsubscript{1α} concentration following recirculation.

When the brain concentrations of PGF\textsubscript{2α} and PGE\textsubscript{2} were plotted against ICBF, it became clear that the apparently random brain PG concentrations have a particular topographic arrangement, as shown in Figure 3. Namely, 5 minutes after recirculation, both PGs were most pronouncedly increased in brain samples with ischemic ICBF values of 15–25 ml/100 g/min. They subsequently decreased to the control value after 2 hours of recirculation. By contrast, the postischemic increase in the concentration of these PGs was relatively small in brain samples with ischemic ICBF values of <15 ml/100 g/min. In brain samples with ischemic ICBF values of near 0 ml/100 g/min, postischemic increase in PG concentrations did not take place.

Our results indicate that postischemic brain concentrations of these PGs are prominently increased in the formerly perifocal area, but not so much in the focal area. One possible explanation of this rather suppressed synthesis of these PGs in focal areas could be the oxygen deficiency in the tissue, suggested by the no-reflow phenomenon or postischemic hypoperfusion.
Although saline perfusion could affect the brain concentration of 6-keto-PGF\(_{1\alpha}\), it is still of interest that the brain concentration of 6-keto-PGF\(_{1\alpha}\) was significantly increased in the focal area immediately after recirculation. Since endothelial cells are more resistant to an ischemic insult than are neuronal cells,\(^{23,24}\) it might be assumed that these different cells exhibit different patterns of cyclooxygenase activation following recirculation.

The brain concentration of any PG examined showed no linear correlation with ICBF either during MCA occlusion or after recirculation. Therefore, it seems unlikely that regional brain concentrations of vasoconstrictive PGs such as PGE\(_2\) and PGF\(_{1\alpha}\) exert significant influences on ICBF. Because of the saline perfusion method we employed, no conclusion could be drawn about the effects of the regional brain concentrations of 6-keto-PGF\(_{1\alpha}\) or TXB\(_2\) on ICBF.

Controversies still exist as to whether PGs and prostaglandin endoperoxidases are related to edema formation in ischemic brain injury. Our present results, in which homogeneous increases of PGF\(_2\) and PGE\(_2\) concentrations in prolonged ischemia and prominent increases of these PGs following recirculation were revealed, suggest at least some participation of these PGs or the cyclooxygenase pathway in edema formation.

**References**


**Key Words** • cerebral blood flow • prostaglandins • cats
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