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# TRANSIENT CEREBRAL ISCHEMIA AND BRAIN PROSTAGLANDINS

Robert J. Gaudet and Lawrence Levine

Department of Biochemistry, Brandeis University
Waltham, Massachusetts 02154

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## SUMMARY

Prostaglandin levels were measured in gerbil brain during cerebral ischemia for up to 2 hours, and during reperfusion after various periods of transient ischemia. The levels of $PGF_{2\alpha}$ and its metabolite (13,14-dihydro-15-keto-$PGF_{2\alpha}$) were low and did not change during ischemia. However, $PGF_{2\alpha}$, 13,14-dihydro-15-keto-$PGF_{2\alpha}$, $PGE_2$, and thromboxane $B_2$ increased during reperfusion after brief episodes of ischemia. Indomethacin or aspirin inhibited this increase. Animals pretreated with indomethacin recovered more rapidly and were more active during reperfusion than those without treatment.

## INTRODUCTION

Normal brain tissue contains only small amounts of prostaglandins. Yet, when brain tissue is traumatized, prostaglandin levels can increase considerably (1); this is probably a result of activation of phospholipase $A_2$, which hydrolyzes cellular phospholipids and releases membrane bound arachidonic acid, a substrate for prostaglandin biosynthesis. Phospholipase $A_2$ activity can be induced by ischemia (2) and the finding of elevated levels of prostaglandin (PG) $F_{2\alpha}$ and $PGE_2$ in cerebro-spinal fluid of patients suffering from stroke (3,4) suggests excessive production and accumulation of prostaglandins in ischemic brain. Such an accumulation of prostaglandins in cerebral tissue may contribute to the pathogenesis of stroke in several ways: (a) The administration of prostaglandins has been shown to cause stupor and catatonia in animals (5,6) so that a substantial increase of prostaglandins in ischemic brain may further impair neuronal function. (b) Many of the prostaglandins are potent vasoactive substances that could contribute to ischemia-induced disorders of the cerebral circulation (7-12). (c) Brain edema is a serious and sometimes fatal complication of stroke (13) and certain prostaglandins or prostaglandin endoperoxides, have been implicated as promoters of inflammation and edema formation in injured tissue (14).

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We have investigated the effects of cerebral ischemia on the levels of prostaglandins in brain tissue using the Monogolian gerbil (Meriones unguiculatus) as an experimental model for stroke. Bilateral occlusion of both common carotid arteries (CCAs) causes severe ischemia of the cerebral hemispheres in these animals because of an incomplete circle of Willis (15,16).

## MATERIALS AND METHODS

Gerbil treatment and brain preparations. Groups of 4 to 7 adult male and female (40-60 g) gerbils were sacrificed under liquid nitrogen at intervals of 5, 15, 30, 60 or 120 minutes after bilateral common carotid artery (CCA) occlusion. Occlusion was accomplished by the application of an aneurysm clip to each carotid artery while the animals were briefly anesthetized with ether. Sham-operated animals, in which the CCAs were exposed but not occluded, were sacrificed immediately and 30 minutes after the operation and used as controls. For reperfusion studies, groups of gerbils, occluded for 5, 15, or 30 minutes, were sacrificed at intervals of 5, 15, 45 and 105 minutes after removal of the vari-angle aneurysm clips (Codman Surgical Instruments, Randolph, MA.) to allow reflow (reperfusion). Additional groups of animals were sacrificed at 5 and 45 minutes of reperfusion after brief, 10 and 60 second, periods of occlusion. Occlusion for 10 and 60 seconds was accomplished by simultaneously crimping the CCAs using loops of suture passed around each artery. This provided more precise control of the duration of occlusion than was possible using the aneurysm clips, which had to be applied and removed one-at-a-time. Occlusion was confirmed visually and blood flow resumed through the arteries after release of occlusion.

The cerebral hemispheres were removed while still frozen and the brain stem and prominent venous sinuses were trimmed-off. Each cerebrum was weighed and then homogenized in 2.5 ml of cold 100% ethanol using a polytron homogenizor (the tissue was not permitted to thaw before homogenization) The samples were centrifuged and the supernatant fluids dried under a nitrogen stream. After reconstitution to 2.5 ml of tris buffer and acidification to pH 3.1, each sample was extracted twice with anhydrous ether. The organic phase was separated and dried under nitrogen and redissolved in 1.0 M tris buffer (pH 7.4), containing 0.1% gelatin. The samples were stored at -20°. Using this procedure, recovery of [$^3$H]$PGE_2$ and [$^3$H]$PGF_{2\alpha}$ added to the homogenates was consistently between 40 and 60%.

For measurement of the effect of indomethacin treatment on post-ischemia behavior of gerbils, groups of animals were subjected to bilateral CCA occlusion for 5 minutes or to a sham-operation as described above. Immediately upon reperfusion (or 5 minutes after sham-operation) the animals were placed in a clear plastic cage (dimensions 25 x 42 x 10 cm) which was covered with a wire top. The number of times in each five minute period of reperfusion the animals crossed a line which bisected the cage was scored. Once in the cage the animals were not disturbed and extraneous noise was held to a minimum. Animals were given I.P. injections of indomethacin (0.5 mg) or vehicle (propylene glycol) approximately 30 minutes prior to surgery.

Measurement of prostaglandins. $PGF_{2\alpha}$, $PGE_2$, 13,14-dihydro-15-keto-$PGF_{2\alpha}$ and 13,14-dihydro-15-keto-$PGE_2$ were measured by radioimmunoassay as described previously (17,18). In the thromboxane RIA, the [$^3$H]thromboxane $B_2$ anti-thromboxane $B_2$ reaction is inhibited 50% by 0.028 ng thromboxane $B_2$. $PGD_2$ cross-reacts 0.15%, and $PGE_2$ and $PGF_{2\alpha}$ cross-react less than 0.1% (19).

Materials. Indomethacin was donated by Merck, Sharp and Dohme, Rahway, N.J.; dexamethasone was obtained from Sigma Chemical Co., St. Louis, MO. and acetylsalicylic acid from Aldrich Chemical Co., Milwaukee, WI.

## RESULTS AND DISCUSSION

The effect of ischemia for up to 2 hours on prostaglandin levels in brain is shown in Figure 1A. There were no significant changes in the levels of $PGF_{2\alpha}$ or its metabolite, 13,14-dihydro-15-keto-$PGF_{2\alpha}$. $PGE_2$ levels were reduced slightly, but only after 120 minutes. The release of arachidonic acid and other fatty acids from cellular lipids is known to occur rapidly after decapitation-induced ischemia in rat brain (20,21). Probably, arachidonic acid also is released after the induction of ischemia in gerbil brain. The brain content of prostaglandins or its metabolites should increase if the released arachidonic acid has been metabolized. Depletion of tissue oxygen, a substrate necessary for the cyclo-oxygenation of arachidonic acid (2 moles of $O_2$ are needed for each mole of prostaglandin produced), may explain this lack of an increase in prostaglandin levels [the induction of complete ischemia in brain causes depletion of brain tissue $O_2$ within one minute, the result of rapid utilization of available $O_2$ for brain-energy production (22)].

The effect of transient cerebral ischemia on the levels of prostaglandins in the brain was striking; reperfusion was accompanied by increases in $PGF_{2\alpha}$, 13,14-dihydro-15-keto-$PGF_{2\alpha}$ and $PGE_2$ after all but the shortest (10 second) period of occlusion (Fig. 1B). These increases occurred rapidly; prostaglandin levels were elevated at five minutes. In those animals occluded for 5, 15 or 30 minutes, prostaglandin levels remained elevated at 15 and 45 minutes of reperfusion and decreased to normal levels after 105 minutes. Only 13,14-dihydro-15-keto-$PGF_{2\alpha}$ concentrations were significantly higher than controls after 105 minutes.

The following considerations can explain this rapid post-ischemic increase of prostaglandins. As a result of ischemia, deacylation of phospholipids by phospholipase $A_2$ causes a release of arachidonic acid, but oxygenation of arachidonate is inhibited because of the lack of oxygen. Upon res-

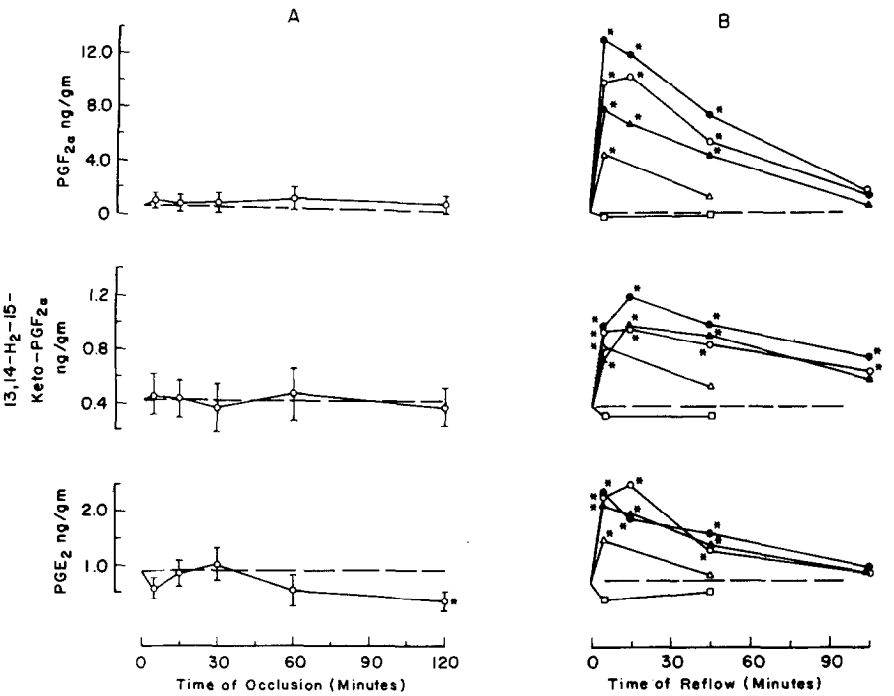


Figure 1. A) Effect of time of occlusion on $PGE_2$, 13,14-dihydro-15-keto-$PGF_{2\alpha}$, and $PGF_{2\alpha}$ levels in the brain. Values are mean ± standard deviation. Broken lines indicate mean of sham-operated control values. B) Effect of time of reperfusion on $PGE_2$, 13,14-dihydro-15-keto-$PGF_{2\alpha}$, and $PGF_{2\alpha}$ levels in the brain. Symbols indicate mean value during reperfusion after occlusion for 10 seconds (□); 1 minute (Δ); 5 minutes (●); 15 minutes (○); and 30 minutes (▲). Standard deviations have been omitted for clarity. *Statistically significant from sham-operated values $p < 0.05$ by analysis of variance.

toration of tissue $O_2$ during reperfusion, the arachidonic acid is rapidly converted to endoperoxides by cyclo-oxygenase and then to the prostaglandins. Prostaglandin levels were not increased after a 10 second period of occlusion; the deacylation of brain lipids and release of arachidonic acid probably requires a period of ischemia longer than 10 seconds. The increase of prostaglandins during reperfusion after 60 seconds of occlusion was less than after longer periods (Fig. 1B), suggesting that the release of arachidonic acid is not maximal after one minute of ischemia.

The levels of $PGF_{2\alpha}$ increased approximately 50-fold during reperfusion after a 5 minute period of occlusion, whereas the levels of $PGE_2$ increased 3-5 fold. $PGI_2$ levels, measured as 6-keto-$PGF_{1\alpha}$, also were determined. In

the RIA for 6-keto-$PGF_{1\alpha}$, 6-keto-$PGF_{1\alpha}$ is measured by inhibition of the heterologous [$^3$H]$PGF_{1\alpha}$ anti-6-keto-$PGF_{1\alpha}$ reaction: 0.14 ng of 6-keto-$PGF_{1\alpha}$ inhibits 50% binding; $PGF_{1\alpha}$ cross-reacts 44%, but $PGF_{2\alpha}$, $PGD_2$, and $PGE_1$ cross react less than 1% (19). The levels of 6-keto-$PGF_{1\alpha}$ in brains after 5 minutes of reperfusion following 5 minutes of occlusion increased 8.5 fold over the levels found after sham-operation. The differences in $PGE_2$ and $PGF_{2\alpha}$ content may reflect the conversion of $PGE_2$ to $PGF_{2\alpha}$ by prostaglandin 9-keto-reductase activity (23) present in brain. When measured at 5 minutes of reperfusion, prostaglandin levels were higher after 5 minutes of occlusion than after 15 or 30 minutes. This suggests that prostaglandin biosynthetic mechanisms are impaired after more prolonged periods of ischemia. After occlusion for 5 to 30 minutes, prostaglandin levels did not return to normal until 105 minutes after reperfusion had begun. This slow return of $PGF_{2\alpha}$ to normal levels, along with low levels of $PGF_{2\alpha}$ metabolite, probably reflects the low 15-hydroxydehydrogenase activity in brain tissue (1,24). The eventual return of prostaglandin levels to normal values with prolonged reperfusion is probably due to vascular washout, or diffusion into cerebro-spinal fluid combined with low metabolic activity.

The post-ischemic increase of brain prostaglandin levels could be blocked by some of the substances known to inhibit prostaglandin synthesis (Table 1). Indomethacin (0.5 mg) was the most potent; it inhibited $PGF_{2\alpha}$ accumulation approximately 93%. Aspirin (10.0 mg) also inhibited, but dexamethasone (0.5 mg) was not effective. The levels of thromboxane $B_2$ were also measured and found to be increased during reperfusion (Table 1). Indomethacin and aspirin, but not dexamethasone, inhibited this increase.

Intra-cerebral administration of prostaglandins is known to induce behavioral depression (5,6). To evaluate whether there is relationship between behavioral and endogenous levels of prostaglandins, groups of gerbils were subjected to cerebral ischemia for 5 minutes and their locomotor activity recorded during reperfusion. As shown in Figure 2, there was an initial latent period of inactivity from 0-15 minutes in both occluded groups. With longer reper-

TABLE 1: THE EFFECT OF INDOMETHACIN, ASPIRIN, AND DEXAMETHASONE ON $PGE_2$, $PGF_{2\alpha}$ AND THROMBOXANE $B_2$ ACCUMULATION IN GERBIL BRAIN AFTER 5 MINUTES OCCLUSION AND 5 MINUTE REPERFUSION

| Pretreatment | 5 Minute Occlusion 5 Minute Reperfusion | Number | $PGF_{2\alpha}$[§] | $PGE_2$[§] | Thromboxane $B_2$ |
|---|---|---|---|---|---|
| | | | ng/gram tissue[†] | | |
| Sham operated control | - | 4 | .29 (.08) | .48 (.11) | <0.02 |
| None | + | 4 | 15.65 (1.63) | 1.75 (.10) | 0.36 (.02) |
| Propylene Glycol* | + | 5 | 15.70 (1.99) | 1.94 (.11) | 0.37 (.03) |
| Dexamethasone, 0.5 mg* | + | 4 | 12.06 (1.98) | 1.30 (.16)** | 0.34 (.03) |
| Aspirin, 10.0 mg* | + | 4 | 2.94 (.68)‡ | 1.01 (.21)‡ | 0.15 (.02)‡ |
| Indomethacin, 0.1 mg* | + | 4 | 7.89 (.99)‡ | 1.14 (.38)‡ | 0.22 (.03)‡ |
| Indomethacin, 0.5 mg* | + | 4 | 1. 4 (.46)‡ | .65 (.12)‡ | <0.02 |

*0.1 ml intra-peritoneal injection 30 minutes before surgery; dexamethasone, aspirin and indomethacin were dissolved in propylene glycol. Aspirin was not completely dissolved in the propylene glycol.
§Data from animals shown in Fig. 1 are not included.
†Mean values, standard error in parenthesis.
**Statistically different from propylene glycol ($p < 0.05$) by Student's t-test.
‡Statistically different from propylene glycol ($p < 0.01$) by Student's t-test.

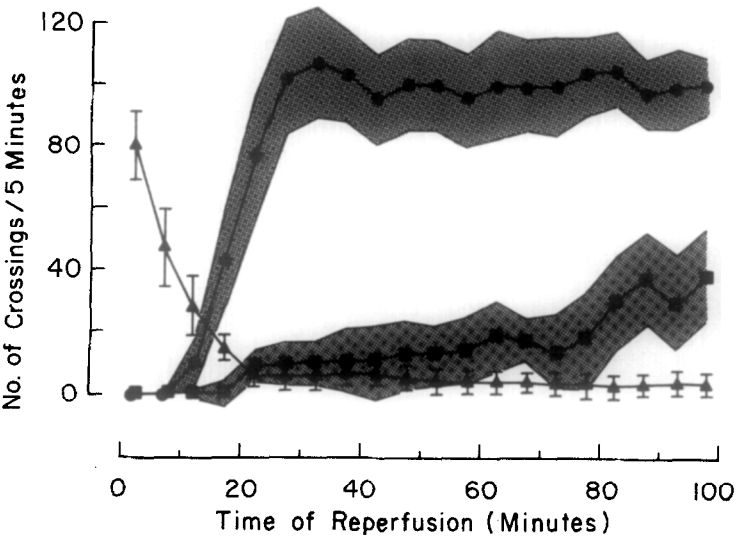


Figure 2. The effect of indomethacin treatment on post-ischemic behavior of gerbils. (▲) Sham-operated animals without indomethacin (N=5); (■) occluded animals without indomethacin (N=8); (●) occluded animals with indomethacin (N=5). Indomethacin-treated sham-operated animals (N=5) demonstrated an activity pattern similar to non-treated sham-operated animals and have been omitted for purposes of clarity. From 15 to 100 minutes, the activity of indomethacin-treated occluded animals was significantly higher ($p < 0.05$, Student't T-test) than that of non-treated occluded animals. Shaded areas represent standard errors.

fusion, the indomethacin-treated gerbils became much more active than the untreated group and remained so during the entire period of observation. Although more active, the behavior of these indomethacin-treated animals could not be considered normal. When compared to sham-operated animals it was apparent that occluded indomethacin-treated animals exhibited sustained hyperactivity. Indomethacin-treated-sham-operated or sham-operated animals were active initially; then their activity decreased as they became accustomed to the enclosure, slept, or spent time in localized areas. In contrast, the indomethacin-treated-occluded animals persistently shuttled back and forth, did not exhibit the curiosity of normal animals, and were easily startled by movement or noise. This hyper-behavior may reflect changes in brain neurotransmitter levels. Brain monoamine neurotransmitter levels become altered during the

course of transient cerebral ischemia in the gerbil [serotonin levels rapidly decrease during ischemia and do not recover during the initial period of reperfusion (25)].

Blood flow to the brain is known to be altered after episodes of transient ischemia. The "no-reflow phenomenon" (impaired re-establishment of flow after brief interruption of circulation to the brain) has been reported in several experimental animals (7-9). Conversely, post-ischemic hyperemia also has been reported (10-12). The accumulation of prostaglandins may contribute to these alterations in blood flow since several of the arachidonic acid metabolites are vasoactive. For example, an increase in the vasoconstrictors, $PGF_{2\alpha}$ and thromboxane $A_2$, as was observed in the present study may contribute to impaired flow during reperfusion. On the other hand, the potent vasodilator, prostacyclin, measured by its stable product, 6-keto-$PGF_{1\alpha}$, also increases during reflow and could contribute to hyperemia.

An increase of brain levels of prostaglandins in response to brief periods of ischemia may contribute to vascular changes seen during migraine headache. The prodrome phase of migraine is associated with a decrease in cerebral blood flow whereas cerebral blood flow increases during the subsequent headache phase (26). Pain occurring during the headache phase is thought to be the result of excessive vasodilation. Recently it has been suggested that ischemia as severe as that occurring during stroke, may occur during the prodrome phase of migraine (27). If a brief period of ischemia does occur during the prodrome phase, then an accumulation of vasodilating prostaglandins around cerebral vessels during reperfusion could contribute to the headache phase by potentiating vasodilation.

Our results demonstrate an accumulation of prostaglandins in brain during reperfusion, but not during ischemia. However, incomplete ischemia (when flow is not totally shut-off but is decreased enough to impair brain function) could result in prostaglandin synthesis if residual flow provides enough $O_2$ for cyclo-oxygenation of arachidonic acid. Further, if ischemia were confined

to a localized area, arachidonic acid could diffuse from the ischemic area into bordering non-ischemic areas and be converted to the prostaglandins. Inhibition of prostaglandin synthesis may be beneficial in the treatment of stroke in humans by alleviating behavioral deficits and improving brain blood flow.

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