Are Prostaglandins Involved in Experimental Ischemic Edema in Gerbils?

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SUMMARY Sixty-five male gerbils, divided into 3 groups, were used in this study, in which focal brain specific gravity, taken as a measure of edema, was compared to the corresponding focal cerebral blood flow using the hydrogen washout technique. Extracranial unilateral or bilateral carotid ligation was performed and one hour later the animal was sacrificed. When focal blood flow was less than 20 ml/100 g/min, edema developed and increased with progressive ischemia, reaching maximal values at 5–7 ml/100 g/min. In the zero flow situation there was no edema. Pretreatment of the other 2 groups with indomethacin or dexamethasone, did not prevent edema formation at flows of 20–12 ml/100 g/min, but considerably reduced the edema previously noted at low flows (5–7 ml/100 g/min). The drugs did not affect the decreased flow in the ischemic area. We conclude that prostaglandins, released by membrane disruption, are involved in the development of ischemic edema.

Experimental work suggests that "ischemic brain edema" has cytotoxic and vasogenic features. The early accumulation of water is presumed to be intracellular, and, if the ischemia progresses, a vasogenic component develops with increasing permeability of the blood-brain barrier. The reasons for these changes are still not clear. It may be that 2 separate pathophysiological effects are progressing simultaneously, or they may be sequential aspects of the same basic mechanism, the degree of one component depending on the severity of the other. Following damage to cell membranes, Wolf has emphasized the release of prostaglandins. Yamamoto and Pickard have shown that prostaglandins are highly vasoactive and their precursor, arachidonic acid, has been shown to produce edema. It might be presumed, therefore, that increasing ischemia eventually leads to cell membrane breakdown and release of vasoactive substances which, in turn, aggravate the situation and increase the amount of edema produced.

We described in a previous paper, an experimental stroke model in the gerbil in which we studied the relationship between the focal cerebral blood flow (rCBF) and brain edema as judged by brain specific gravity. The Mongolian gerbil, because of the incompleteness of the circle of Willis, has been extensively used to study the histological changes associated with ischemia, edema formation, changes in the blood-brain barrier and focal cerebral blood flow which developed following extracranial carotid ligation. Our initial studies showed that edema developed when the regional blood flow was less than 20 ml/100 g/min and increased with increasing ischemia to a maximum at blood flows around 7 ml/100 g/min. Blood flows less than this were associated with little or no change in the brain’s specific gravity. To discover if the prostaglandins, or their precursors, are involved in the post-ischemic edema formation, we repeated our studies having pretreated the animals with indomethacin, a well known inhibitor of prostaglandin synthesis. Another approach to the same problem would be to "stabilize" the cell membrane, as well as prevent the action of these vasoactive substances; in another part of this same experiment, the animals have been pretreated with dexamethasone.

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Material and Methods

Measurements

Local cerebral blood flow (rCBF) was evaluated by the hydrogen clearance technique and washout curve analyzed by the "initial slope" technique. We considered zero flow to exist only when there was no clearance from the tissue and no change with subsequent resaturation of the animal with hydrogen.

Brain specific gravity measurements are those described by Nelson using the brombenzene kerosene columns prepared to produce a graded and continuous gradient from top to bottom of the column. Four to 6 samples of grey matter 1 mm³, carefully dissected with the operating microscope from the tissue surrounding each electrode, but excluding the area perforated by the hydrogen electrode, were measured. The dissection was carried out in a brombenzene kerosene mixture and samples transferred immediately to the measuring column which was kept at a constant temperature by a water jacket. Before each series of measurements, known specific gravity droplets were inserted to check the linearity of the column which was only used if the correlation coefficient of linearity was UNITY. Measurements of the samples in the column were made exactly 3 min after insertion. No samples were stored for later estimation as we have shown that the brombenzene kerosene solution alters brain samples after 30 min immersion and makes them appear less dense. Using these rigorous techniques of dissection and measurement, the variation in specific gravity from a particular region was less than 0.5%.

Preparation and Procedure

Sixty-five male mongolian gerbils (Meriones unguiculatus) weighing 40 to 60 g were used for this study. The animals were anesthetized with pentobarbital 60 mg/kg body weight injected intraperitoneally. Other anesthetic agents have been used but the level of anesthesia produced is inconstant. Although barbiturates affect cerebral metabolism, a constant level of anesthesia was considered more important so as to maintain steady blood pressure and constant level of anesthesia was considered more important so as to maintain steady blood pressure and blood gases. The surgical preparation has been previously described, but is here briefly outlined. Four 1 mm long 100 µm diameter, Teflon coated platinum iridium electrodes were inserted into the grey matter through burr holes drilled on each side of the sagittal suture, 2 in front of the coronal suture and 2 behind and fixed with methylmethacrylate. A 32 gauge polyethylene catheter was inserted into the left femoral artery and connected to a blood pressure transducer (Statham P23 G). Small blood samples (0.2 mls) were removed for blood gas measurement on a Radiometer ABL 3 blood gas analyzer. No more than 0.8 ml were removed from one gerbil and volume was carefully replaced with saline to avoid hypovolemia. In most, only the right common carotid artery was occluded, but in a smaller series a bilateral occlusion was performed. No animal was used in any part of the experiment whose blood gas analysis, blood pressure or temperature was abnormal. Control observations were performed on all the animals and following this they were divided into 3 groups (see below). Exactly one hour after occlusion the animals were sacrificed, the brain quickly removed and placed in a brombenzene-kerosene solution. There was a separate series of animals in each of the 3 groups which had the complete surgical procedure, but did not have carotid occlusion. This was done to take into account the effects of surgery and, in those animals pretreated with indomethacin or dexamethasone, to evaluate the effects of these drugs on cerebral blood flow and brain specific gravity. The 3 groups of animals were:

a) Untreated Group

Fifteen gerbils had a right common carotid artery occluded and 4 had bilateral carotid occlusion. Six other animals were used as controls having the surgical preparation but without carotid occlusion.

b) Indomethacin Pretreated

Indomethacin, 3 mg/kg body weight in 0.2 ml, was injected intraperitoneally into the gerbils after control observations. The drug is rapidly absorbed, crosses the blood-brain barrier and inhibits 80% of all prostaglandin synthesis in blood and brain. Indomethacin had been prepared in a phosphate buffer adjusted to pH 7.4 immediately before the injection. The effects on rCBF, blood pressure and heart rate were studied and one hour after the intraperitoneal injection, a right common carotid occlusion was performed in 10 animals, and a bilateral occlusion in 4 animals. The procedure then followed the standard preparation. Six animals were used as 'controls.' In these animals surgical preparation was identical, indomethacin was injected, the animals were studied for a period of 2 hours without carotid occlusion and specific gravity measurements carried out as before. Two other gerbils were injected with the phosphate buffer solution, with no indomethacin added, to exclude an effect on blood flow or brain specific gravity of the buffer solution.

c) Dexamethasone Pretreated

Dexamethasone 2.5 mg/kg body weight in 0.2 ml was injected intraperitoneally and the procedure was as before. In 8 animals, a right carotid occlusion was performed, 4 underwent a bilateral carotid occlusion and 6 animals were used as controls.

Results

Untreated Group

We have described already the relationship of flow and edema following unilateral and bilateral carotid occlusion. Some points to compare the effect of indomethacin or dexamethasone pretreatment (fig. 1) are summarized.

With the animal spontaneously breathing, the mean control cerebral blood flow was 29.2 ± 4.9 ml/100
FIGURE 1. This figure illustrates the relationship between brain specific gravity and rCBF following carotid occlusion in our experimental groups. The specific gravity, measured after unilateral or bilateral occlusion, was related to the corresponding rCBF measured during the occlusion and means specific gravity for 2 ml/100 g/min intervals rCBF were obtained for each group. In the untreated group edema begins at 20 ml/100 g/min and is maximal at 5–7 ml/100 g/min. Those pretreated with indomethacin or dexamethasone show considerable reduction in the brain water content at extremely low flows in comparison to the untreated group.

Unilateral carotid occlusion resulted in a flow of 12.9 ± 5.8 ml/100 g/min on the ipsilateral side, with no significant change in flow one hour after the occlusion. In the contralateral hemisphere the group postischemic flow (25.6 ± 6.9 ml/100 g/min) was not significantly different from the control values. After bilateral carotid occlusion the mean blood flow was 2.8 ± 1.3 ml/100 g/min initially and this fell to 0.5 ± 0.5 ml/100 g/min an hour later. There was no difference in frontal and parietal CBF values.

The control specific gravity was 1.0500 ± 0.0004 and one hour after carotid occlusion it was 1.0462 ± 0.0017 on the ipsilateral side. Bilateral occlusion resulted in a specific gravity measurement of 1.0495 ± 0.0008, not significantly different from the controls.

Correlating individual flow with individual specific gravity measurements we noted the following points. The decrease in brain specific gravity was closely related to postocclusive flow of the region (fig. 1). The increase in brain water content, started at CBF less than about 19 ml/100 g/min, reached the maximum levels at flow values of 7 ± 2 ml/100 g/min, and, with CBF values close to zero, there was no change in specific gravity from the control measurement. For flows in the regions of 7 ± 2 ml/100 g/min the decrease in specific gravity was more than expected from the projection of the exponential line of flow and edema which began at 20 ml/100 g/min (fig. 1).

Indomethacin Pretreated

The indomethacin treatment did not result in a significant change in blood pressure and blood gases, but CBF was reduced from 28.9 ± 3.2 ml/100 g/min to 24.6 ± 5.4 ml/100 g/min one hour later. For those animals with unilateral occlusion the mean immediate postocclusive flow in the ipsilateral hemisphere was 15.6 ± 5.2 ml/100 g/min and one hour later 18.1 ± 7.9 ml/100 g/min. On the contralateral side there was the same wide range of flow as found in the untreated group. In the animals with bilateral occlusion, the mean blood flow from all regions was not significantly different from the untreated group but there was a significantly higher flow in the parietal as compared to the frontal regions (p < 0.01).

In the animals injected with indomethacin, without occlusion, the grey matter specific gravity was 1.0485 ± 0.0002, significantly lower (p < 0.001) than the untreated controls. One hour of occlusion resulted in a further decrease in specific gravity (1.0471 ± 0.0008) in the hemisphere ipsilateral to the occlusion, while on the contralateral side and in those with bilateral occlusion the animals did not show any change in specific gravity from the six control animals pretreated with indomethacin. There was a lower brain specific gravity at control blood flows, but at CBF values around 19–20 ml/100 g/min there was a similar decrease in specific gravity to that noted in the untreated group and increasing edema noted with decreasing flow. This reached the lowest levels at flows of 13 ± 1 ml/100 g/min; from which point there was an "inflexion in the curve" to reach similar specific gravity measurements as found in indomethacin treated controls (figs. 1, 2). Indomethacin, but not the phosphate buffer, decreased control values of specific gravity, but prevented the
marked reduction in specific gravity noted at low flows.

**Dexamethasone Pretreated Group**

The control rCBF for this group (before dexamethasone) was 28.0 ± 7 ml/100 g/min and one hour after the injection: 24.1 ± 5.6 ml/100 g/min; there was no change in blood pressure and blood gases. After unilateral carotid occlusion the mean initial flow in the ipsilateral hemisphere was 10.1 ± 3.4 ml/100 g/min and the final 13.8 ± 6.5 ml/100 g/min showing no difference from the untreated animals. In the contralateral side the initial post occlusive flow was significantly less (p < 0.05) than the untreated animals, but this difference disappeared one hour later, when the flow was 24.7 ± ml/100 g/min. After bilateral occlusion the rCBF was similar to the untreated model of bilateral occlusion.

There was no change in the grey matter specific gravity as compared with the untreated controls, in the group given dexamethasone without carotid occlusion. Unilateral carotid occlusion resulted in a grey matter specific gravity, of 1.0478 ± 0.0006 on the ipsilateral side, significantly (p < 0.02) higher than the untreated group.

There was no difference between the specific gravity of control gerbils, results from the contralateral hemisphere and from those animals with bilateral occlusion (fig. 3).

The rCBF was related to the specific gravity as before (figs. 1, 3). In this group there was no change in control animals as compared with the untreated groups, so that at "normal" flows the specific gravity results are identical to those for the untreated gerbils. Again, when the flow was less than 19 ml/100 g/min, the specific gravity decreased to lowest values around 7 ml/100 g/min. Even at these low flows there was considerably less edema as judged by specific gravity than in the untreated group, and the curve showed the previously described inflexion, albeit markedly attenuated.

**Discussion**

What is emerging from recent work suggests that the degree of edema associated with ischemic lesions in brain is related to the blood flow in the ischemic region. Waltz pointed out the value of experimental models in the study of cerebral ischemia and stressed the threshold that appears with cerebral blood flow values of less than 20 ml/100 g/min. Symon et al. has also shown that edema begins at blood flows less than 20 ml/100 g/min in the baboon and we have reported similar findings in gerbil cerebral ischemia.

What causes this variation in water content during ischemia is not clear. It is no surprise that in the no flow situation there can be no accumulation of water. In the area of moderate ischemia (10-20 ml/100 g/min) the increasing water content, as judged by decreasing brain specific gravity, might be due to ischemia and/or anoxia affecting the ionic pump. The marked increase in edema at blood flows around 7 ± 2 ml/100 g/min suggested to us that there is another mechanism affecting the development of brain edema at these low flows. Astrup et al. described the massive efflux of potassium at similar flow values and they suggested that at this level of flow there was damage to the cell membrane from which the cell could not recover; less severe ischemia, while preventing brain electrical activity, had the potential for survival. The question, therefore, that we raised was, if the cell membrane, damaged or disrupted at these low flow values, released substances such as arachidonic acid, and if these substances, taking part in the cascades of phospholipid metabolism, were responsible for the edema production?

To test this hypothesis, we repeated our original experiments of extracranial carotid occlusion in the gerbil, but pretreating the animals with either indomethacin or dexamethasone. If prostaglandins or related compounds are involved in the edema formation, then the amount of edema produced in our model should be less.

Brain tissue has been shown to be able to synthesize prostaglandins and thromboxanes under normal conditions. This biosynthesis, however, from endogenous precursors, is particularly active in damaged tissue. Patients with subarachnoid hemorrhage and stroke show an increase of PGE₂ and PGF₂α in cerebrospinal fluid. Prostaglandins, on the other hand, are thought to play a role in cerebrovascular homeostasis. Indomethacin, at the dosage that we used, causes about 80% of prostaglandins synthesis inhibition in rat brain after one hour with a half life of 32 hours. The ratio between plasma and brain is constant and the inhibition of PGE₂ and PGF₂α is at about the same level. Although we are not aware of the relevant pharmacological data in the gerbil, we assume that the activity and metabolism in the rat and gerbil are similar.

Corticosteroids have been shown to reduce cerebral
edema associated with brain tumors or metastases. In several models of brain edema, developing after acute cerebral ischemia, dexamethasone has been shown to be effective. It has been postulated that the mechanism of steroid action in cerebral edema may be explained by the "membrane stabilizing" effect, resulting in protection from the lysosomal enzyme release and from a free radical injury. Corticosteroids also inhibit the prostaglandin synthesis by blocking the release of arachidonic acid from the cells, without acting, as indomethacin does, on the cyclo-oxygenase system.

Waltz's group pointed out that after middle cerebral occlusion in cats, dexamethasone was ineffective in reducing the size of the cerebral infarct. In the same model the drug was effective in modifying cerebral edema, in infarcted tissue more than in ischemic tissue when evaluated 2 days after the occlusion. This was believed to be due to its effect on the transendothelial distribution of proteins. In addition, dexamethasone influences also the early ischemic edema in cats and in the gerbil. Whatever its effect on established strokes, dexamethasone was used by us only to inhibit prostaglandin synthesis. The reduction in edema with pretreatment is not comparable to treatment of the established stroke.

Pretreatment with either drug did not prevent the development of edema at moderate ischemia, but did effectively reduce treatment occurring at very low flows. The increased water content in control animals pretreated with indomethacin is difficult to explain. It may be that fluid accumulates due to loss of vasoreactivity, or it may be that the pretreatment with indomethacin allows the accumulation of arachidonic acid in the normal brain producing a slight degree of edema.

How may these facts be linked? The formation of edema at flow values between 20 and 12 ml/100 g/min may not be related to products of cell membrane damage. At flows less than 10 ml/100 g/min, the amount of edema is considerably less following pretreatment, suggesting these substances are involved. The fact that both inhibition of their synthesis, at 2 different levels, or "membrane stabilization" reduced the edema formation seems to be strong evidence implicating the prostaglandin series or allied compounds in edema formation at these low flow values.

There are several experiments which are particularly germane to this study. Hoppe et al. showed that there was less water in infarcted tissue than in ischemic tissue after having pretreated the animals with dexamethasone. This is in keeping with our findings. Long has recently shown that in the cold injury model the amount of edema around the cold injury could be considerably reduced by the excision of the cold lesion itself. This may well indicate that the damaged tissue is the source of the substances which encourage edema formation. Assays of brain tissue prostaglandins following transient carotid occlusion, followed by reperfusion in the gerbil, have demonstrated marked rises in all functions, particularly in $F_2$ and $E_4$; we have also confirmed these observations. Thus, there is mounting evidence of the role of prostaglandins in ischemic edema.

To summarize: in ischemic brain edema, the amount of edema is dependent on the residual flow in the ischemic area. Edema begins at about 20 ml/100 g/min, reaches a peak at 7 ml/100 g/min and there is no edema in the no flow situation. Inhibition of prostaglandin synthesis or membrane stabilization does not affect edema formation at moderate ischemia (12-20 ml/100 g/min) but does affect edema formation at flows lower than this. Indomethacin or dexamethasone does not affect the decreased flow in the ischemic area. We conclude that in severe ischemia, prostaglandins released by cell membrane disruption, are implicated in edema formation.

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