Effect of Aminophylline on Postischemic Edema and Brain Damage in Cats

M. Seida, MD, H.G. Wagner, MD, K. Vass, MD, and I. Klatzo, MD

We attempted to ameliorate postischemic edema and brain tissue injury in cats by administering aminophylline to reduce the reactive hyperemia that supposedly aggravates both these sequelae. Forty-one cats were subjected to 1 hour of middle cerebral artery occlusion and were killed after 3 hours, 3 days, or 14 days of recirculation; one half of the cats received 0.916 ml/kg of a 25 mg/ml solution of aminophylline by infusion at a constant rate via the femoral vein starting 10 minutes before release of the occlusion and continuing for 5 minutes after initiation of recirculation; the other half received saline. Regional cerebral blood flow was monitored by the hydrogen clearance method and water content was evaluated by specific gravity measurements after 3 hours of recirculation; the status of the blood–brain barrier was assessed with Evans blue tracer. Morphologic observations were carried out in cats killed after 3 or 14 days of recirculation. Aminophylline-treated cats killed after 3 hours of recirculation showed significantly reduced hyperemia and edema and no leakage of Evans blue, which was present in all untreated cats killed after 3 hours or 3 days of recirculation. Morphologic observations revealed conspicuously more severe ischemic brain tissue damage in the untreated than in the aminophylline-treated cats after 3 and 14 days of recirculation. Our studies indicate the beneficial effect of administration of aminophylline in the amelioration of postischemic edema and brain tissue injury, which is presumably achieved by reduction of reactive hyperemia.

(Stroke 1988;19:1275–1282)

The pathomechanism of reactive hyperemia, which frequently follows release of a major cerebral artery occlusion, has not been entirely clarified. Generally, it has been assumed that accumulation of extracellular H\(^+\), associated with lactacidosis,\(^1\) and efflux of K\(^+\) from ischemic cells\(^2\) as well as extracellular hypocalcemia constitute the main factors in producing relaxation of cerebral smooth muscle cells and vascular dilatation. However, since the dynamic characteristics of these ionic changes and hyperemia appear to be not well synchronized, involvement of some other vaso-dilatatory agents, especially of the potent cerebrovascular dilator adenosine, seems likely.

Similarly unclear has been the role of reactive hyperemia in the pathophysiology of ischemic injury. A number of authors have suggested that reactive hyperemia may have a beneficial effect. Among them, Cuypers and Matakas\(^3\) considered that sustained hyperemia prevented postischemic "no-reflow" and intracranial hypertension. Osburne and Halsey\(^4\) found that absence of postischemic hyperemia closely correlated with failure of electroencephalographic recovery and eventual brain death. Similarly, Hossmann et al\(^5\) reported that hyperemia, by improving the postischemic blood circulation after global ischemia, was essential for the functional recovery of the brain.

Conversely, several investigators have reported a deleterious effect of reactive hyperemia on the development of postischemic edema and brain tissue injury. Thus, Mchedlishvili et al\(^6\) strongly suggested that reactive hyperemia promotes edema. Heiss et al\(^7\) found that in cats after middle cerebral artery (MCA) occlusion a spontaneous, marked hyperemia was associated with pronounced edema and severe brain infarcts. A direct relation between reactive hyperemia and the ensuing edema was clearly outlined by Kuroiwa et al\(^8\) in studies demonstrating biphasic opening of the blood–brain barrier (BBB) to serum proteins after MCA occlusion in cats. These studies indicated that the prompt onset of marked reactive hyperemia is associated with the first opening of the BBB and leakage of serum proteins, inducing vasogenic edema. Further support for this notion has been provided by studies of Ting et al\(^9\) showing that reduction of reactive

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Received February 2, 1988; accepted April 8, 1988.
hyperemia by hypovolemia could prevent opening of the BBB and could significantly reduce postischemic edema and ischemic brain tissue damage compared with control animals that were exposed to ischemia of similar intensity followed, however, by marked reactive hyperemia.

Since prevention of hyperemia by hypovolemia has proved to be rather erratic under experimental conditions, we have searched for a pharmacologic agent that could affect postischemic hyperemia and could be of potential clinical value. Considering the possibility that adenosine plays a major role in the development of reactive hyperemia following recirculation of ischemic territory, we tested aminophylline, an adenosine receptor blocker, to ascertain whether it could reduce reactive hyperemia and thus ameliorate postischemic edema and brain tissue injury.

**Materials and Methods**

Forty-one cats of either sex (average wt 3.1 kg) were subjected to 1 hour of transorbital MCA occlusion. The cats were injected with 30 mg/kg i.m. ketamine hydrochloride and 0.05 mg/kg i.m. atropine sulfate. In 21 cats the femoral artery was cannulated for continuous monitoring of arterial blood gases, hematocrit, and arterial blood pressure. The femoral vein was also cannulated for administration of drugs and Evans blue. All 41 cats were intubated and placed prone with the head fixed in a Kopf stereotactic frame. Rectal temperature was monitored continuously. All surgical wounds were infiltrated with procaine.

In 21 cats used for evaluation of regional cerebral blood flow (rCBF) and water content changes measured by specific gravity (SG), after the administration of drugs and Evans blue. All 41 cats were subjected to 1 hour of transorbital MCA occlusion. The cats were injected with 30 mg/kg i.v. a-chloralose and 2% halothane-49% nitrous oxide-49% oxygen supplied by endotracheal tube. The cephalic vein was cannulated for administration of drugs and Evans blue. Removal of the eyeball in these cats was associated with blepharorrhaphy, avoiding any laceration of the palpebral conjunctiva, to have sterile conditions in the operative field. The skin incision over the orbit was sutured, and artificial ventilation was stopped when spontaneous breathing became reestablished, which usually occurred 2–3 hours after the induction of anesthesia. The cats were returned to their cages, and antibiotics were given to prevent infections. These 20 cats were scheduled to be killed after 3 or 14 days of recirculation, five untreated and five aminophylline-treated cats each day.

Aminophylline (USP, Abbott Laboratories, North Chicago, Illinois) was administered to 21 cats (11 in the rCBF/SG group and 10 in the morphologic group) by infusing 0.916 ml/kg of a 25 mg/ml solution (which corresponds to approximately 0.1 μmol/g as theophylline) at a constant rate via the femoral vein starting 10 minutes before release of the MCA occlusion and continuing for 5 minutes after initiation of recirculation. The 20 untreated cats (10 in the rCBF/SG group and 10 in the morphologic group) received a similar infusion of saline.

To assess permeability of the BBB, five untreated and five aminophylline-treated cats in the rCBF/SG group received 2 ml/kg i.v. of 2% Evans blue 30 minutes before release of the occlusion. All 20 cats in the morphologic group received Evans blue 1 hour before sacrifice.

To measure SG, the brains of the 21 rCBF/SG cats were removed immediately after sacrifice, immersed in a water-treated kerosene gradient column, and cut coronally 15 mm anterior to the interaural plane, which corresponded to the plane of electrode insertion. Approximately 1-mm³ fragments were excised from the caudate (three samples); suprasylvian, ectosylvian, and sylvian gyri (four samples); and the adjacent white matter (two samples) on both the ischemic and the nonischemic sides. The samples were placed directly into the gradient column for determination of SG according to the method described.

rCBF was evaluated in the 21 rCBF/SG cats by the hydrogen clearance method before, during, and up to 3 hours after MCA occlusion. Hydrogen inhalation lasted 3–15 minutes. The first 40 seconds of hydrogen clearance was excluded to allow for clearance of pulmonary and arterial hydrogen. rCBF was calculated as $F = \frac{A}{(0.693/\lambda 	imes 100)}$, where $F =$ blood flow in milliliters per 100 grams per minute, $A =$ brain/blood partition coefficient for hydrogen (assumed to be 1), 0.693 = natural logarithm of 2, and $T_{1/2}$ is the time in minutes for the polarographic current to indicate half-desaturation; rCBF was based
on a regression line fitted to a series of timed values plotted on semilog paper. Usually, the plotted values indicated a single slope; rarely, there was evidence for a second slope, in which case rCBF was based on the slope associated with the 2-minute value.\textsuperscript{15-17} rCBF was determined twice before the occlusion and after 10 minutes, 30 minutes, 1 hour, and 2 hours 45 minutes of recirculation. In untreated cats, rCBF was also determined after 20 minutes of recirculation.

The brains of the 20 cats in the morphologic group were perfused with 4% buffered paraformaldehyde at 140-150 mm Hg pressure and were sectioned coronally 15 mm anterior to the interaural plane, as in the rCBF/SG group cats. The 5-mm blocks were embedded in paraffin, and alternate 10-μm sections were stained with cresyl violet or hematoxylin and eosin. The areas examined under the light microscope covered the territory of the MCA and included primarily the caudate and the cerebral cortex of the suprasylvian, ectosylvian, and sylvian gyri.

Results

Physiologic variables, which included mean arterial blood pressure, body temperature, Pco\textsubscript{2}, Po\textsubscript{2}, pH, and hematocrit, in the 21 rCBF/SG group cats remained within normal ranges during and after MCA occlusion. There were no significant differences between untreated and aminophylline-treated cats (Table 1).

Clinically, in the morphologic group two of the 10 untreated cats scheduled to be killed after 14 days died after 3 and 5 days of recirculation, whereas all 10 aminophylline-treated cats were alive at the time of their sacrifice. All five untreated cats killed after 3 days of recirculation showed hemiparesis on the right side, whereas hemiparesis was noticeable in only two of the five aminophylline-treated cats.

TABLE 2. Regional Cerebral Blood Flow in Ipsilateral Caudate Nucleus of 21 Cats Before, During, and After 1-Hour Middle Cerebral Artery Occlusion

<table>
<thead>
<tr>
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<th>Untreated</th>
<th>Aminophylline-treated</th>
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<td></td>
<td>n</td>
<td>Before</td>
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<tr>
<td>MABP (mm Hg)</td>
<td>10</td>
<td>42.0 ± 14.6</td>
</tr>
<tr>
<td>Pco\textsubscript{2} (mm Hg)</td>
<td>11</td>
<td>41.5 ± 15.7</td>
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Values are mean ± standard deviation ml/100 g/min.
*tp<0.001, p<0.01 compared with untreated cats.
FIGURE 1. Regional cerebral blood flow (rCGF) in cats measured in caudate nucleus ipsilateral to middle cerebral artery (MCA) occlusion. ●, untreated (n=10); ○, aminophylline-treated (n=11). *p<0.001, **p<0.01 compared with untreated. Specific gravity: untreated 1.0412±0.0045; aminophylline-treated 1.0453±0.0015*.

SG after 3 hours of recirculation is presented in Table 3. Aminophylline-treated cats revealed significantly less edema (p<0.001) in the ischemic brain regions on the side of the occlusion than untreated cats, although water content in the aminophylline-treated cats was significantly higher (p<0.001) in the occluded than the unoccluded side.

Observations in the 30 cats receiving Evans blue concerning changes in permeability of the BBB revealed that Evans blue was occasionally extravasated in a narrow zone surrounding the track of the polarographic electrode, clearly related to the trauma of electrode insertion. Other small areas of blue immediately adjacent to the site of surgical injury associated with transorbital MCA occlusion were of clearly traumatic origin. Among cats killed after 3 hours of recirculation, none of the five aminophylline-treated cats showed Evans blue extravasation in the ischemic region, whereas all five untreated cats revealed Evans blue leakage in the caudate and three of five showed blue in the cerebral cortex subjected to ischemia (Table 4). Among cats killed after 3 days of recirculation, one of five aminophylline-treated cats showed Evans blue leakage in the caudate and cerebral cortex, whereas four of five untreated cats showed staining in the caudate and all five revealed Evans blue extravasation in the cortex.

Among cats killed after 14 days of recirculation, neither untreated nor aminophylline-treated cats revealed any Evans blue leakage.

The morphologic observations after 3 or 14 days of recirculation were carried out in a blind fashion, without the examiner's knowledge of the treatment. The microscopic changes were classified into four categories according to intensity of ischemic injury: 0, no or very minimal ischemic changes; +, distinct but moderate ischemic injury; ++, pronounced ischemic damage but preservation of general tissue structure; and ++++, very severe ischemic damage with almost complete destruction of neuronal elements or necrotic infarction.

More precise criteria for rating changes in the caudate and the cerebral cortex subjected to ischemia in cat brains after 3 days of recirculation were used. In the caudate, + denoted evident ischemic injury of small neurons expressed as hyperchromasia or loss of Nissl substance and good preservation of large neurons (Figure 2A); in the cerebral cortex, + referred to areas of slight ischemic neuronal changes characterized by loss of Nissl substance and occasional presence of dark or vacuolated neurons with loss of Nissl substance. In both the

| Table 3. Specific Gravity Measurements in Brains of 21 Cats 3 Hours After 1-Hour Middle Cerebral Artery Occlusion |
| --- | --- | --- | --- |
| n | Side | Caudate nucleus | Middle cerebral artery territory |
| --- | --- | --- | --- | --- |
| Untreated | 10 | Occluded | 1.0412±0.0045 | 1.0436±0.0028 | 1.0458±0.0016 |
| | | Unoccluded | 1.0472±0.0007 | 1.0472±0.0010 | 1.0469±0.007 |
| Aminophylline-treated | 11 | Occluded | 1.0453±0.0015* | 1.0453±0.0017* | 1.0460±0.0009 |
| | | Unoccluded | 1.0469±0.0006 | 1.0465±0.0009 | 1.0467±0.0011 |

Values are mean±standard deviation.
*p<0.001 compared with occluded side of untreated cats.
caudate and the cortex, + change was associated with mild vascular response characterized by the presence of occasional inflammatory cells of both the leukocytic and monocytic varieties (Figure 2B). In the caudate, + + referred to severe ischemic damage of the small neurons, which were either pyknotic or very pale or "shadow-like," whereas the large nerve cells were still relatively well preserved (Figure 2C). In the cerebral cortex, + + denoted presence of numerous dark or pale neurons with loss of Nissl substance and distorted processes; the vascular network was very conspicuous in both structures, standing out by intense staining of its cellular elements, with presence of occasional perivascular inflammatory cells (Figure 2D). In both the cortex and the caudate, + + + referred to almost complete disappearance of neuronal elements; the vascular reaction consisted of vigorous proliferation of endothelial cells and the formation of new channels associated with numerous macrophages and reactive microglia cells. Vascular and macrophagic reactions were particularly conspicuous in the marginal areas, those surrounding areas of infarction.

In the cats killed after 14 days of recirculation, the criteria for the various degrees of ischemic injury were slightly different. In the caudate, + denoted a recognizable loss of Nissl substance in the small neurons, whereas large neurons appeared to be well preserved. In the cerebral cortex, + referred to occasional small foci of glial and vascular reactivity associated with paleness and reduction in the number of neurons in those regions. In both the caudate and the cerebral cortex, + + was assigned when the neuronal injury or loss was more pronounced and the glial reaction and vascular proliferation were more intense; + + + signified complete destruction of the parenchyma associated with intense organization of infarcted tissue and intense vascular proliferation associated with numerous gitter cells (Figure 2E). Some infarcted areas showed colliquative necrosis, with no recognizable cellular structures present.

As shown in Table 5, there was a clear difference in intensity of injury between untreated and aminophylline-treated cats. In cats killed after 3 days of recirculation, this difference was especially apparent in the cerebral cortex, which in four of five untreated cats showed severe ischemic injury. Only one aminophylline-treated cat revealed a complete, sharply circumscribed focus of infarction in the caudate; otherwise, the aminophylline-treated cats revealed no lesions of above + + intensity.

The comparison of brain tissue changes in untreated and aminophylline-treated cats killed after 14 days of recirculation also indicated a conspicuous difference. The brains of four of five untreated cats revealed extensive areas of very severe ischemic injury. Among the four, the caudate and cortex of two cats, which were removed soon after death and fixed by immersion, showed extensive areas of infarction with colliquative necrosis. In comparison, only one aminophylline-treated cat revealed a circumscribed area of infarction in the caudate; other aminophylline-treated cats revealed moderate or no changes.

**Discussion**

The involvement of adenosine in postischemic reactive hyperemia is supported by several considerations. Since concentrations of adenosine triphosphate become depleted during cerebral ischemia, a secondary accumulation of adenosine should be expected and, indeed, a rapid, progressive elevation of adenosine concentration has been demonstrated during 60 seconds of ischemia. The effect of adenosine as a potent vasodilator was observed directly after topical applications, and there are indications that adenosine might be an important mediator of metabolic regulation of cerebral blood flow. Methylxanthines such as theophylline and aminophylline are known to act as cyclic adenosine monophosphate phosphodiesterase inhibitors, to stimulate release of catecholamines, to mobilize calcium, and to be effective adenosine receptor blockers. These various actions are dose-dependent, however, and at concentrations of $\leq 10^{-4}$ M in the CSF their effect may be selectively limited to blocking adenosine receptors. Using theophylline at effective concentrations in the CSF (in the range of $10^{-4}$ M), Winn et al demonstrated a selective effect of this compound as an adenosine receptor blocker and observed a significant reduction of hypoxic hyperemia. Similarly, inhibition of the vasodilatory effect of perivascularly applied adenosine by theophylline was observed by Wahl and Kuschinsky and Ko et al.

The effects of theophylline or aminophylline in protracted cerebral ischemia or in clinical cases of stroke have not been entirely clear. Aminophylline has been used for a long time in the immediate treatment of cerebral infarction, and its action was
FIGURE 2. Morphologic changes in cats after 3 or 14 days of reperfusion following middle cerebral artery occlusion. Cresyl violet stain, bar=30 μm. A. Caudate of aminophylline-treated cat killed after 3 days. Large cells are well preserved; small cells show pyknosis or loss of Nissl substance (+). B. Cerebral cortex of aminophylline-treated cat killed after 3 days. Small vein on left shows slight infiltration by monocyctic cells; neurons show loss of Nissl substance (+). C. Caudate of untreated cat killed after 3 days. Small cells are recognizable mostly in shadow form; large cells are still well preserved (++). D. Cerebral cortex in aminophylline-treated cat killed after 3 days. Neurons are either pyknotic or chromatolytic; blood vessels reveal increased cellularity (++). E. Cerebral cortex of untreated cat killed after 14 days. Intense proliferation of vascular elements and accumulation of macrophages; brain parenchyma is no longer recognizable (+++).
interpreted as related to the "inverse intracerebral steal phenomenon" associated with constriction of normal brain vessels, whereas this response was abolished and blood flow was even increased in the ischemic areas.24 Similar findings with aminophylline were observed in MCA occlusion in cats by Regli et al.25 A suggestion of inverse steal, that is, vasoconstriction in normal brain tissue associated with no such effect or increased blood flow in ischemic tissue, was found by Olsen et al.26 with application of theophylline in stroke patients.

It must be mentioned that the above investigations concerning the effect of methylxanthines in cerebral ischemia refer primarily to application of these compounds in unrelieved arterial obstructions, and it can be assumed that the reactive hyperemia that promptly follows release of arterial occlusion may have different pathophysiologic features.

Although autoregulation may still be impaired during hyperemia, autoregulation recovers much sooner than the cerebrovascular responsiveness to changes in Paco2 and the effect of aminophylline given at the time of release of the MCA occlusion was clearly evident in significant reduction of rCBF in ischemic areas compared with our untreated cats. Although in previous preliminary investigations27 a promising beneficial effect on postischemic edema and tissue injury was obtained using theophylline, in our present investigation aminophylline was chosen in view of better control of its administration provided by infusion of an aqueous solution at constant rate for 15 minutes around the time of release of the MCA occlusion. We did not measure drug concentrations. The total dose of aminophylline (0.1 μmol/g as theophylline) corresponds to an average concentration of approximately 10^{-4} M in the CSF.

Our study provides further evidence that postischemic reactive hyperemia plays a significant role in the opening of the BBB associated with extravasation of serum proteins, leading to intensification of postischemic edema and brain tissue injury. It is also apparent from our observations that reduction of hyperemia, achieved by application of aminophylline at the dose and time given, resulted in significant amelioration of both edema and tissue damage. The effect of aminophylline on hyperemia was evident from rCBF measurements revealing that, in spite of very similar rCBF values before and during occlusion, the aminophylline-treated cats showed markedly reduced hyperemia for up to 1 hour compared with untreated cats. Parallel to reduction of hyperemia, the aminophylline-treated cats showed significantly less edema after 3 hours of recirculation. At that time, aminophylline completely prevented opening of the BBB to Evans blue, whereas all untreated cats showed leakage of the tracer. Extravasation of Evans blue was also markedly different between untreated and aminophylline-treated cats killed after 3 days of recirculation.

The morphologic evaluation of tissue damage was difficult in view of the variable intensity of ischemic injury in different regions and some uncertainty in grading according to our subjective criteria. Nonetheless, the difference in intensity of ischemic injury was obvious when comparing incidences of the most severe injuries (+ + +) in untreated and aminophylline-treated cats.

Our studies may be relevant to a clinical situation of recirculation of previously ischemic territories, which has become increasingly common in neurosurgical procedures involving cerebral blood vessels. Also, it should be kept in mind that embolic infarction, which is the most common form of a clinical stroke, is characterized by frequent relocation of the original embolus, thus creating an intense hyperemia in previously ischemic areas.28

The other point to be kept in mind is the ever-changing character of postischemic circulatory disturbances. Thus, therapeutic efforts in clinical situations should be carefully and appropriately adjusted to the phases of pathophysiologic mechanisms prevailing at a given time. Under such circumstances, with rapid development of diagnostic technology including the possibility of sustained monitoring of rCBF, application of methylxanthines in certain clinical situations associated with cerebral ischemia may deserve consideration.

Acknowledgments
We are indebted to Mrs. I. Fenton and Mrs. J. Lohr for their expert technical assistance and to
Mrs. H. Mittelman for editorial assistance in preparing this manuscript.

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Stroke. 1988;19:1275-1282
doi: 10.1161/01.STR.19.10.1275
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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