Effect of Enhanced Capillary Activity on the Blood–Brain Barrier During Focal Cerebral Ischemia in Cats

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We hypothesize that enhanced activity of capillary Na,K-ATPase promotes Na⁺ influx into the brain and causes early edema formation in focal cerebral ischemia. The pharmacologic suppression of brain capillary Na,K-ATPase as a means to ameliorate edema formation was examined using the middle cerebral artery occlusion model in 36 cats. With the help of a catheter inserted into the middle cerebral artery, the ischemic brain area was directly perfused with 10⁻⁵ M ouabain. Perfusion was maintained as intermittent 15-second pulse injections given every 5 (n=6) or 2 (n=6) minutes. By this method, the naturally occurring circulatory conditions during ischemia were not altered. Four hours after ischemia, the cortical specific gravity at each of six locations over the ischemic area was compared with the corresponding ischemic blood flow measured by the H₂ clearance technique. The results show that ouabain perfused every 2 minutes significantly ameliorated edema formation compared with six control cats perfused with Krebs-Ringer solution. In a separate series of experiments, the Na⁺ flux across the blood–brain barrier was studied by injecting ²²NaCl together with an intravascular reference (cobalt-57-labeled microspheres 15 μm in diameter) into the ischemic area. The brain uptake index of ²²Na was markedly increased in the ischemic cortex of six control cats; ouabain treatment in six cats suppressed the increase of Na⁺ influx. The results support our hypothesis that brain capillary Na,K-ATPase activity increases during early focal ischemia, leading to enhanced Na⁺ together with H₂O flux across the blood–brain barrier. (Stroke 1989;20:1260–1266)

Focal cerebral ischemia causes instantaneous astroglial swelling with accumulation of brain Na⁺ and H₂O, that is, cytotoxic edema.¹⁻³ This early development of ischemic brain edema has been explained based upon the osmotic gradient,⁴ hydrostatic pressure,⁵ and changes in ionic environment.⁶ However, it is not known whether the net increase in tissue Na⁺ concentration is a result of increased Na⁺ influx or of reduced clearance in the ischemic brain tissue,⁷ and relatively little attention has been paid to the movement of Na⁺ across the blood–brain barrier (BBB). Here, we focus on the role of capillary Na⁺-K⁺-ATPase in moving Na⁺ into brain tissue under normal and ischemic conditions.

Normal ionic (in particular, Na⁺ and K⁺) conditions are maintained by Na,K-ATPase, which contributes to cell volume regulation; this is known as Donnan’s pump-and-leak hypothesis.⁸ In brain capillaries, Betz et al⁹ proposed a polarity of Na⁺ movement across the BBB. Na,K-ATPase located at abluminal sites pumps Na⁺ from the blood into the brain in exchange for K⁺. Thus, the extracellular concentration of K⁺ is regulated in a narrow range under conditions of normal cellular activity. The massive shift of K⁺ into the extracellular space that occurs quickly after cerebral ischemia would alter such mechanisms of ion pumping.

There are several reasons that brain capillaries retain their structure and function during the early period of ischemia, when neuronal function ceases. The cerebral capillary endothelium constitutes a tough barrier to ion movement and is relatively resistant to ischemic insult.¹⁰,¹¹ Endothelial cells do
not develop histologic abnormalities during the early stage of ischemia, except for some microvilli formation. Particularly in focal ischemia, capillary endothelium is where the residual blood supply first comes into contact with, and easily receives energy sources from, the blood.

The Na⁺:K⁺ ratio in ischemic brain tissue during the early ischemic period is approximately 2,3 which coincides with the pump ratio of Na⁺,K⁺-ATPase. Further, there is increased activity of capillary Na⁺,K⁺-ATPase in vitro when cells are exposed to low concentrations of hydroperoxide of arachidonic acid. Thus, during the early period of ischemia, persistent functioning of capillary Na⁺,K⁺-ATPase may contribute to the flux of Na⁺ and H₂O across the BBB into the brain. In support of this, recent studies have shown enhanced activity of brain14-16 or capillary17 Na⁺,K⁺-ATPase both during the early stages of ischemia and after reperfusion. These findings contrast with those of studies showing decreased brain Na⁺,K⁺-ATPase activity following ischemia.18-21 This discrepancy may be explained by differences in the duration of ischemia and the tissue compartments examined.

We aimed to inhibit with ouabain the presumed enhanced activity of capillary Na⁺,K⁺-ATPase. We employed a new method to target brain capillary function, a local intravascular perfusion technique within the ischemic area. We also examined the Na⁺ flux across the BBB.

Materials and Methods

Adult mongrel cats weighing approximately 3 kg were anesthetized with 2-4% halothane/N₂O/O₂ under mechanical ventilation. In each cat, both femoral arteries and a femoral vein were cannulated for monitoring arterial blood pressure, for blood gas measurements, and for the administration of fluids. The left orbital contents were removed, and the middle cerebral artery (MCA) was exposed at its origin. Six platinum electrodes were implanted within the cortical layer inside the ischemic focus for measuring cerebral blood flow (CBF) by the H₂ clearance technique. End-tidal O₂ and CO₂ concentrations were continuously monitored, and all parameters such as blood pressure and arterial blood gas concentrations were kept within the normal range (i.e., blood pressure >100 mm Hg, PaO₂ >100 mm Hg, PaCO₂ 30-35 mm Hg, pH 7.3-7.5). Baseline CBF (before induction of ischemia) was measured several times, and the MCA was occluded at its origin by diathermy. Immediately upon occlusion, a polyethylene catheter (o.d. 1.2 mm, i.d. 0.8 mm) with a tapered tip was inserted through an arteriotomy in the MCA just distal to the occlusion. The MCA catheter was then fixed with biologic fast glue (Aron-Alpha) and was connected to both the pressure transducer (to measure MCA stump pressure) and to the perfusion apparatus. The perfusate, Krebs-Ringer solution (millimolar composition Na⁺ 145, K⁺ 4.3, Cl⁻ 132, Ca²⁺ 2.4, Mg²⁺ 1.2, HCO₃⁻ 26, H₂PO₄⁻ 1.2, SO₄⁻ 1.2, glucose 10; pH 7.40, 290 mosm) aerated with 95% O₂/5% CO₂ was perfused (for 15 seconds) intermittently (every 5 or 2 minutes) with a hydrostatic loading pressure of 20 mm Hg more than the MCA stump pressure. In a preparatory investigation, this pressure was sufficient to perfuse the entire MCA territory. The perfusion duration and intervals were controlled with a magnetic on-off valve connected to a handheld computer. Intermittent perfusion lasted from 30 minutes after MCA occlusion until 30 minutes before the cat was killed; ischemia lasted 4 hours. To confirm homogeneity of the perfusion, carbon black was perfused in separate cats.

Krebs-Ringer solution alone was the perfusate for the two control perfusion groups and 10⁻³ M ouabain was dissolved in Krebs-Ringer solution for the two ouabain perfusion groups. The ionic composition and pH of the Krebs-Ringer solution did not change after the addition of ouabain. Higher concentrations of ouabain (10⁻¹ and 10⁻⁴ M) killed the cats due to its cardiotoxicity. CBF was measured just after MCA occlusion but before perfusion and every hour during perfusion. Groups of cats investigated were 1) no-perfusion control (n=11), 2) control perfusion every 5 minutes (n=7), 3) control perfusion every 2 minutes (n=6), 4) ouabain perfusion every 5 minutes (n=6) and 5) ouabain perfusion every 2 minutes (n=6). The total volume perfused during the 3 hours was 10 ml for every 5-minute perfusion and 25 ml for every 2-minute perfusion.

Brain tissue samples were immediately taken from each electrode site after the cat was killed with intravenous saturated KCl, and specific gravity was measured with the aid of an automated microgravimetric apparatus as previously reported. At least two samples weighing 10-15 mg each were measured at every electrode site. CBF during ischemia, a mean of several measurements during perfusion, was correlated with subsequent edema formation (specific gravity).

In 12 other cats, Na⁺ flux across the BBB was investigated by injecting ²²NaCl into the MCA catheter after either control (n=6) or ouabain (n=6) perfusion every 2 minutes during 4 hours of ischemia. ²²NaCl (in the amount of 5 μCi) together with cobalt-57-labeled microspheres 15 μm in diameter (in the amount of 10 μCi) was injected as a bolus over 15 seconds, and the cats were killed 5 seconds later with intravenous saturated KCl. The volume of radioactive solution injected was 0.5 ml. Cortical tissue samples weighing approximately 50 mg were taken from each electrode site for CBF measurements, and their radioactivity was counted by an automatic scintillation counter (Autowell Gamma System, Aloka type JDC 761R, high voltage 1.16 KV, window ±30) for gamma-ray emission. Brain uptake index (BUI) according to Oldendorf was calculated as BUI=(²²Na/²⁷Co)brain−(²²Na/²⁷Co)injectate and correlated with ischemic CBF during perfusion.
Analysis of variance was used to compare multiple groups, and Wilcoxon’s nonparametric test was used to compare two groups; $p<0.05$ was considered significant.

**Results**

Intra-arterial perfusion with carbon black stained the area in and around the MCA territory homogeneously. On coronal sections, carbon black penetrated the full thickness of the cortical layer beyond the periphery of the MCA branches near the midline (Figure 1). Also in a previous investigation, angio-

**FIGURE 1.** Coronal sections of cat brain after perfusion with carbon black within ischemic area caused by middle cerebral artery occlusion. Note very homogeneous distribution of dye.

graphic demonstration of the perfused area confirmed that a hydrostatic loading pressure of approximately 20 mm Hg was enough to cover the entire territory of the MCA. Thus, our technique guarantees equal distribution of even particulate solutions within the ischemic brain tissue so that the use of radiolabeled microspheres for measuring BUI is justified.

Sequential changes of ischemic CBF were compared (Figure 2). Generally, ischemic CBF before and during perfusion did not differ from that of the no-perfusion control group. Further, ischemic CBF did not differ between interval groups within a perfusion treatment. Therefore, the degree of ischemia was approximately equal in all groups.

In all groups, brain specific gravity decreased when ischemic CBF was <25–30 ml/100 g/min (Figure 3). Below this threshold, specific gravity decreased parallel to decreases in ischemic CBF. Maximum edema was observed when CBF was nearly 0 ml/100 g/min.

Because there were apparently different CBF thresholds for edema formation (25–30 ml/100 g/min) and the development of infarct (<15 ml/100 g/min), changes in specific gravity were compared in two CBF ranges, severe ischemia (<15 ml/100 g/min) and moderate ischemia (15–30 ml/100 g/min; Figure 4). Compared with no-perfusion controls (data not shown), there was a slight tendency to exacerbation of edema formation due to control perfusion with Krebs-Ringer alone in both CBF ranges. When ouabain was perfused every 5 minutes, specific gravity did not differ from that for the corresponding control perfusion group. However, ouabain perfused every 2 minutes significantly ameliorated edema formation in both severe and moderate ischemia.

In control cats in which Na+ flux was investigated, BUI increased when ischemic CBF was <30 ml/100 g/min (Figure 5); BUI increased greatly as
ischemic CBF decreased. By contrast, after ouabain perfusion this massive increase of BUL was markedly inhibited, indicating suppression of Na⁺ flux across the BBB.

Discussion
We have found that brain microvessels retain their function and contribute to edema formation by actively pumping Na⁺ into the brain; the major mediator is capillary Na,K-ATPase. Underlying mechanisms should be sought in relation to cell volume regulation, cerebral energy metabolism, and lipid peroxidation at capillary endothelium–astroglia interfaces.

Our intermittent local perfusion technique has an obvious advantage in that BBB function can be
fusion. Ouabain (A, $10^{-5}$ M) clearly inhibits this increase in BUI except in tissues with near-zero flow.

![Graph](image)

**Figure 5.** Scatterplot of brain uptake index (BUI) of $^{22}$Na against intravascular reference, cobalt-57-labeled microspheres in cats after middle cerebral artery occlusion. BUI increases as ischemic blood flow falls below 30 ml/100 g/min in control cats (●) with Krebs-Ringer perfusion. Ouabain (Δ, $10^{-5}$ M) clearly inhibits this increase in BUI except in tissues with near-zero flow.

Cells or the blood stream through Na,K-ATPase activation. Na,K-ATPase activity in the central nervous system (CNS) is not uniform but differs in each compartment such as neurons, astrocytes, and synaptosomes with respect to responses to extracellular $K^+$ concentration and ouabain. $^{25-28}$ Further, microvessels and the choroid plexus also have the capacity to bind ouabain. $^{29}$ Under normal conditions, changes in $K^+$ concentration in the extracellular space are limited to a ceiling of 12 mM. $^{30}$ This seems to be to a large extent due to active incorporation of $K^+$ into glial cells via Na,K-ATPase, which can be maximally activated up to 20 meq/l, whereas its activity in neurons is already saturated at lower $K^+$ concentrations, near physiologic ranges.

Regarding ion channels other than Na,K-ATPase in glial cells, Kimelberg and Bourke $^{31}$ have proposed that $H^+$ is extruded from the glia during intracellular acidosis. This is accomplished through operation of ion antiport systems, that is, $H^+/Na^+$ and $HCO_3^{-}/Cl^-$ antiporters, by which intracellular $H^+$ and $HCO_3^{-}$ are transported outside while extracellular $Na^+$ and $Cl^-$ are taken inside the cell, causing glial swelling. Such ion exchanges represent the cellular mechanism to cope with intracellular acidosis at the expense of volume under physiologic and pathologic conditions. $^{32-37}$

Brain endothelial cells can also influence the normal level of extracellular $K^+$. $^{38}$ The uptake of $K^+$ into isolated brain capillaries increases in parallel with an increase in the extracellular concentration of $K^+$. $^{39}$ Therefore, an increase in extracellular $K^+$ would enhance the activity of the Na$^+$ pump and would increase Na$^+$ influx. Several ion channels may operate at the capillary endothelium on each luminal and abluminal side. $^{40-42}$ But Na,K-ATPase seems to be a major mediator for Na$^+$ influx. $^{43}$ Betz et al. $^{44}$ have claimed that Na,K-ATPase is located on the abluminal side of brain microvessels, but controversy still exists as to whether the enzyme is located only on the abluminal side or on both the luminal and abluminal sides. $^{44}$

There is evidence that such enhanced activity of capillary Na,K-ATPase is mediated by products of lipid peroxidation of arachidonic acid. The activity of microvessel Na,K-ATPase is enhanced by a challenge with 15-HPAA in vitro, whereas it has the opposite effects in synaptosomes. $^{13}$ On the contrary, Lo and Betz $^{45}$ reported reduction of K$^+$ uptake by hydrogen peroxide radicals mediated by inhibition of brain microvessel Na,K-ATPase. These disparate results seem to be explained by differences in the radical species generated and their concentrations because 15-HPAA at high concentrations also inhibits microvessel Na,K-ATPase. $^{13}$

Ouabain has been used to inhibit Na,K-ATPase in various compartments of the CNS such as the neurons, glia, capillaries, and choroid plexus. Na,K-ATPase can be completely inhibited at a concentration of $10^{-3}$ M in vitro, but systemic use at this
concentration is not possible because of its cardio-
toxic effect. In our study, 10⁻⁵ M was the highest 
ouabain concentration cats could tolerate. When 
infused via the cortical artery at high concen-
trations, ouabain also caused glial but not neuronal 
swelling, while arterioles exhibited permeability 
changes. Capillary Na,K-ATPase might be 
involved in vasogenic brain edema induced by cold 
injury. Gazendam et al. observed increases in K⁺ 
concentration and decreases in Na⁺ concentration 
edema fluid caused by intracerebral ouabain 
administration within the edemic focus. Astrup et 
investigated to what extent Na,K-ATPase-
related energy consumption operated in the CNS 
using an isolated brain perfusion model. By the use 
of ouabain and other drugs, they found that Na⁺-K⁺ 
leak–flux mechanisms depended partially upon 
Na,K-ATPase. Intraventricular administration of 
ouabain was reported to reduce cerebrospinal fluid 
formation by 70–80%, and the remaining fluid was 
thought to be of extrachoroidal origin, possibly 
from microvessels. It was, however, not until our 
study that microvessels were challenged directly 
within the ischemic focus. Further astroglial 
swelling was anticipated, the result was reduced 
edema formation paralleled by inhibition of Na⁺ 
flux across the BBB.

We conclude that early cytotoxic edema in cerebral 
ischemia is an active process, or probably an adaptive 
response before being totally governed by irreversible 
necrotic processes. The search for interactions 
between brain capillaries and astroglia should bring us 
a more fundamental rationale for the treatment of 
ischemic brain edema and neuronal damage.

Acknowledgments

For technical assistance, we thank Mrs. Reiko Matsuura and Mr. Aihara, who is at the Isotope 
Center of University of Tokyo Hospital.

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KEY WORDS • blood-brain barrier • brain edema • cats
Effect of enhanced capillary activity on the blood-brain barrier during focal cerebral ischemia in cats.
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Stroke. 1989;20:1260-1266
doi: 10.1161/01.STR.20.9.1260
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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World Wide Web at:
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