

Biphasic Opening of the Blood-Brain Barrier Following Transient Focal Ischemia: Effects of Hypothermia

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ABSTRACT: Objective: Tracer constants (K_i) for blood-to-brain diffusion of sucrose were measured in the rat to profile the time course of blood-brain barrier injury after temporary focal ischemia, and to determine the influence of post-ischemic hypothermia. **Methods:** Spontaneously hypertensive rats were subjected to transient (2 hours) clip occlusion of the right middle cerebral artery. Reperfusion times ranged from 1.5 min to 46 hours, and i.v. ^3H -sucrose was circulated for 30 min prior to each time point (1h, 4h, 22h, and 46h; $n=5-7$ per time point). K_i was calculated from the ratio of parenchymal tracer uptake and the time-integrated plasma concentration. Additional groups of rats ($n=7-8$) were maintained either normothermic (37.5°C) or hypothermic (32.5°C or 28.5°C) for the first 6 hours of reperfusion, and K_i was measured at 46 hours. **Results:** Rats injected after 1.5 – 2 min exhibited a 10-fold increase in K_i for cortical regions supplied by the right middle cerebral artery ($p<0.01$). This barrier opening had closed within 1 to 4 hours post-reperfusion. By 22 hours, the blood-brain barrier had re-opened, with further opening 22 and 46 hours ($p<0.01$), resulting in edema. Whole body hypothermia (28°C - 29°C) during the first six hours of reperfusion prevented opening, reducing K_i by over 50% ($p<0.05$). **Conclusions:** Transient middle cerebral artery occlusion evokes a marked biphasic opening of the cortical blood-brain barrier, the second phase of which causes vasogenic edema. Hypothermic treatment reduced infarct volume and the late opening of the blood-brain barrier. This opening of the blood-brain barrier may enhance delivery of low permeability neuroprotective agents.

RÉSUMÉ: Ouverture biphasique de la barrière hémato-encéphalique suite à une ischémie focale transitoire: effets de l'hypothermie. Objectif: Nous avons mesuré les constantes d'un traceur (K_i) de la diffusion de sucrose du sang vers le cerveau chez le rat afin d'observer l'évolution des dommages subis par la barrière hémato-encéphalique après une ischémie focale temporaire et pour déterminer les effets d'une hypothermie post-ischémique. **Méthodes:** Des rats spontanément hypertendus ont été soumis à une occlusion de deux heures de l'artère cérébrale moyenne par un clip. Le temps de reperfusion variait de 1.5 minute à 46 heures et une perfusion intraveineuse de ^3H -sucrose a été administrée pendant 30 minutes avant chaque évaluation ponctuelle (1h, 4h, 22h, et 46h; $n=5-7$ par évaluation ponctuelle). La constante K_i a été calculée à partir de l'indice de captation du traceur par le parenchyme et de la concentration plasmatique en fonction du temps. Des groupes additionnels de rats ($n=7-8$) ont été maintenus soit à la température normale (37.5°C) ou en hypothermie (32.5°C ou 28.5°C) pendant les 6 premières heures de la reperfusion et K_i a été mesurée à 46 heures. **Results:** Les rats qui ont reçu l'injection après 1.5 – 2 minutes présentaient une augmentation de K_i de dix fois supérieure dans les régions corticales irriguées par l'artère cérébrale moyenne ($p<0.01$). Cette ouverture de la barrière s'était refermée 1 à 4 heures post-reperfusion. À 22 heures, la barrière hémato-encéphalique s'était réouverte, davantage à 22 et à 46 heures ($p<0.01$), ce qui a donné lieu à de l'œdème. L'hypothermie généralisée (28°C - 29°C) pendant les 6 premières heures de la reperfusion a empêché son ouverture, diminuant ainsi la constante K_i de plus de 50% ($p<0.05$). **Conclusions:** L'occlusion transitoire de l'artère cérébrale moyenne provoque une ouverture biphasique importante de la barrière hémato-encéphalique corticale dont la deuxième phase cause de l'œdème. L'hypothermie a diminué la taille de l'infarctus cérébral et l'ouverture tardive de la barrière hémato-encéphalique. Cette ouverture de la barrière hémato-encéphalique peut accroître la distribution d'agents neuroprotecteurs à basse perméabilité.

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Brain capillary walls are distinguished by an endothelial cell layer replete with tight junctions and a scarcity of fenestrae. This blood-brain barrier (BBB) is a specialization which maintains homeostasis of the neuronal micro-environment, limiting blood-to-brain diffusion of hydrophilic molecules. Penetration is largely restricted to lipophilic substances capable of directly traversing endothelial membranes, and to hydrophilic substances such as amino acids and glucose, for which specific membrane carriers exist.^{1,2} BBB drainage, following brain ischemia, leads to the extravascular leakage of plasma proteins and other solutes, resulting in an imbalance with osmotic forces drawing excess

water into the tissue (i.e. vasogenic edema).³ Tissue swelling ensues within the rigid confines of the skull, elevating intracranial pressure with secondary ischemia due to compression of microvasculature, and, ultimately, brain herniation.⁴

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A reproducible rodent model used to study focal brain ischemia involves temporary (e.g. 0.5 to 4 hours, or permanent occlusion of a middle cerebral artery [MCAO model] with tandem occlusion of the ipsilateral common carotid artery).^{5,6,7} Temporary MCAO enables the study of both positive and negative aspects of post-ischemic reperfusion, which occurs clinically, both spontaneously and therapeutically, with the advent of r-tPA, newly licensed as a thrombolytic agent for ischemic stroke in the first three hours. Restoration of blood flow, if early enough, may offer the advantage of reducing neuronal damage and limiting infarct extension and enhances potentially cytoprotective drug delivery. These benefits may be undermined by reperfusion injury to the microvasculature, compromising BBB function, exacerbating edema formation and the inflammatory developments that follow.^{2,3,4} Dynamic changes in BBB permeability, which follow temporary MCAO, are therefore of critical importance.

The objective of this study was to delineate the time course and intensity of the BBB opening after reversible MCAO ischemia in the rat using a method involving a removable, atraumatic clip.⁶ Post ischemic changes in the BBB were studied using a modification⁸ of a radiotracer methodology, which quantifies even minor degrees of BBB opening with a high degree of sensitivity.^{9,10} The radiotracer method used in this study is based on a 2-compartment (plasma/brain) simple diffusion model, which assumes that the amount of ³H-sucrose, which permeates the microvasculature into brain parenchyma, does so in proportion to the time integral of plasma tracer concentration. Normal BBB permeability and opening are indexed in the ratio of parenchymal uptake relative to plasma integral, calculated as the transfer constant, K_i .^{9,10} The effects of delayed hypothermia¹¹ on the biphasic BBB opening were also investigated.

METHODS AND MATERIALS

Male spontaneously hypertensive rats (SHR) weighing 170g – 225g were fasted for 18 hours prior to surgery, but allowed free access to water. Animals were initially anesthetized with 3% halothane, and subsequently maintained on 1 – 2% halothane mixed with 70% nitrogen and 28% oxygen. Body temperature was maintained at $37.5^\circ\text{C} \pm 0.5^\circ\text{C}$ during surgery, by rectal thermistor coupled to a heating lamp.

The method for producing reversible focal ischemia has been fully described.⁶ The right common carotid artery was isolated through a ventral midline neck incision and ligated. A 1cm incision was made perpendicular to and bisecting a line between the lateral canthus and the right eye and the external auditory canal. The underlying temporalis muscle was partially excised. A burr hole, 1 mm in diameter, was drilled 2 – 3 mm rostral to the point of fusion of the zygomatic arch with the temporal bone. Drilling was accompanied by a gentle drip of isotonic saline to prevent warming of the underlying cortex. The dura over the MCA was then cut and retracted. A #1 micro-clip (Codman) was placed on the MCA at a site proximal to the point where it crosses the inferior cerebral vein in the rhinal fissure. The incisions were then closed with wound clips.

Animals were subjected to two hours of ischemia, during which time the anesthesia was discontinued and the animals allowed to regain consciousness. At the end of ischemia, the rats

were briefly re-anesthetized with halothane, the MCA clip removed, and blood re-flow through the MCA visually verified. The wound was sutured closed, and the animal permitted to regain consciousness. Sham groups of animals were treated in the same manner, except the micro-clip was placed on the MCA and then removed immediately. Animals were maintained at a rectal temperature of $37^\circ\text{C} - 38^\circ\text{C}$ for all procedures, except where indicated otherwise in the hypothermic experiments.

Regional transfer constants (K_i) for BBB permeation of ³H-sucrose were measured by a previously published method.^{8,10} modified after ⁹ Measurements were made at different time points after reperfusion of the right MCA. The rats were anesthetized with pentobarbital (65 mg/kg i.p.) and, after cannulation of a femoral artery and vein, ³H-sucrose (NET-341) was injected intravenously (20 $\mu\text{Ci}/100\text{g}$, in 0.5 ml saline). Immediately upon tracer injection, syringe-pump sampling of femoral arterial blood was begun at a constant rate (0.039 ml/min⁻¹) and continued for 30 min. At this point, sampling was stopped and the brain was immediately cleared of intravascular tracer⁸ by perfusing 25 ml saline at 100 – 130 mm Hg pressure through a cannula inserted a few minutes beforehand into the right carotid artery.¹² The rat was decapitated, the brain removed and dissected bilaterally into the cortex (about 180 mm³, representing the complete MCA supply territory), striatum, and hippocampus. Brain samples (weighed) and measured volumes of plasma from the arterial sample were placed in the scintillation vials and solubilized overnight at 37.5°C in 1.3 ml Soluene 350 (Packard Instr.). 10 ml of fluor (HionicFluor) was added to all vials and the samples were counted by liquid scintillation to determine the tracer level in the brain parenchyma (C_{paren} , dpm.g⁻¹) and the time integral of the plasma tracer level ($\int \alpha^{1800} C_{\text{plasma}} dt$, dpm.s.ml⁻¹). The integral was obtained by multiplying the plasma concentration (C_{plasma} , dpm.ml⁻¹) by the circulation time (1800s). The transfer constant (K_i , mL.g⁻¹.s⁻¹) was calculated from the relationship: $K_i = C_{\text{paren}} / \int \alpha^{1800} C_{\text{plasma}} dt$.⁹

In **Experiment 1**, radiotracer studies were carried out at 1, 4, 22, and 46 hours after reperfusion (or 3, 6, 24, and 48 hours after the onset of ischemia) with five animals in each group. In an additional group (n = 5), the radiotracer experiments were initiated within 1.5 – 2 min after reperfusion. In this case only, pentobarbital rather than halothane, anesthesia was induced 15 min before reperfusion for cannulations and clip removal. A group of sham-operated rats (n = 7 total) was studied at 1, 2, 3, 5, and 24 hours after sham occlusion, with one or two rats at each time point. No sham-operated rats were studied at 1.5 - 2 minutes.

In **Experiment 2**, rats underwent mild or moderate hypothermia during the first 6 hours of reperfusion. Ten minutes before the end of 2 hours of MCA occlusion, the rats were lightly halothane anesthetized and were surrounded by bags of crushed ice. This caused body temperature to drop to $32^\circ\text{C} - 33^\circ\text{C}$ at the time that the MCA clip was removed. Rectal temperature was maintained for 6 hours at $32^\circ\text{C} - 33^\circ\text{C}$ for the mild hypothermic group (n=7) or $28^\circ\text{C} - 29^\circ\text{C}$ for the moderate hypothermic group (n=7). Control animals, maintained at $37^\circ\text{C} - 38^\circ\text{C}$, were concurrently studied with their hypothermic peers, such that two normothermic groups were formed (n = 8 for the mild hypothermia controls, and n = 7 for the moderate hypothermia controls). During the six hours, halothane was continued at 0.5 – 0.7% for the hypothermia groups only, as the control group did

Table 1: Regional Transfer Constant (K_i) in MCA Model (Experiment 1)

Variability Group (n)	Right (Mean \pm SD) (mL.g ⁻¹ .s ⁻¹ x 10 ⁶)			Left (Mean \pm SD) (mL.g ⁻¹ .s ⁻¹ x 10 ⁶)		
	Cortex	Striatum	Hippocampus	Cortex	Striatum	Hippocampus
Sham (7)	1.6 \pm 0.8 ^b	1.3 \pm 0.6 ^b	1.7 \pm 0.7	1.9 \pm 0.4	1.3 \pm 0.3	1.9 \pm 0.3
Immediate (5)	17.3 \pm 11.9 ^{abc}	3.4 \pm 1.0 ^a	2.8 \pm 1.0	2.8 \pm 1.0 ^d	2.2 \pm 0.6	3.0 \pm 1.1
RP-1 Hour (5)	5.7 \pm 4.1 ^{ac}	2.4 \pm 1.0	2.3 \pm 0.9	2.3 \pm 0.4 ^d	1.9 \pm 0.8	2.1 \pm 0.4
RP-4 Hours (5)	5.9 \pm 2.9 ^{ab}	2.7 \pm 1.9	2.0 \pm 1.0	3.0 \pm 1.4	2.1 \pm 1.1	2.7 \pm 1.1
RP-22 Hours (5)	13.1 \pm 2.0 ^{abc}	5.3 \pm 1.0 ^{ab}	2.4 \pm 0.5 ^b	2.3 \pm 0.7 ^d	1.6 \pm 0.4 ^d	2.3 \pm 0.9
RP-46 Hours(5)	59.0 \pm 9.3 ^{ac}	16.5 \pm 6.8 ^a	6.0 \pm 2.6 ^a	2.5 \pm 0.8 ^d	2.1 \pm 0.78 ^d	2.7 \pm 0.8

$p < 0.05$ for ANOVA plus Tukey's test.

Within tissues and between treatments: a – different from control (sham stroke) value; b – different from value immediately below.

Within treatments and between tissues: c – different from all other values; d – left side values different from corresponding right side values.

not require anesthesia during reperfusion. Afterwards, these animals were permitted to recover from anesthesia and return to normothermic conditions. Radiotracer experiments were carried out at 46 hours post-reperfusion.

In **Experiment 3**, to assess edema development after two hours of MCA occlusion, rats were sacrificed by decapitation after 4 (n = 5), and 46 (n = 6) hours of reperfusion. Samples of right and left cortex were dissected out and the percentage water content was obtained by weight difference after complete oven drying. The effect of 6 hours post-reperfusion hypothermia was also examined in relation to edema formation. Six rats were subjected to 6 hours of post-reperfusion moderate hypothermia (28°C - 29°C) followed by 40 hours of normothermia (37°C - 38°C).

In **Experiment 4**, neocortical infarct volume was measured in separate animals to determine the effect of hypothermia after MCAO. Rats underwent two hours of ischemia and were then kept either normothermic (n = 6), or immediately underwent 6 hours of mild (32°C - 33°C, n = 6) or moderate (28°C - 29°C, n = 6) hypothermia, as above. Rats were anesthetized and decapitated after 46 hours of reperfusion and the brains removed and frozen. Coronal sections, 20 μ m thick, were cut at -25°C, and every 25th section was saved. The sections were stained with hematoxylin and eosin, and infarct volume and edema were obtained with an image processing system (Image-Pro II). Infarct area of each section was traced, and the total infarct volume was calculated by summing the infarcted area of sequential sections and multiplying the interval thickness between sections. Edema was calculated by comparing the increase in size of the right hemisphere as compared to the left hemisphere.

Statistical analyses used in the transfer constant and edema studies were the ANOVA plus Tukey's test. A two-tailed Student's t test was used to compare cortical infarct volumes in hypothermic versus control animals. A p-value of less than 0.05 was taken to indicate statistical significance. All values are presented as \pm SD.

RESULTS

Experiment 1

Mean regional transfer constants for the six groups of normothermic rats are summarized in Table 1. In 7 sham-stroked rats, radiotracer measurements were initiated at 1, 2, 3, 5, or 24 hours after the sham procedure. Each time period group contained 1 animal, except for the 3 and 24 hour groups, which contained 2 animals each. There were no significant differences between the regional K_i values for cerebral tissues on the right side of the brain (which had undergone a complete surgical procedure, except for MCA occlusion), and those of corresponding territories on the left side. In the 5 experimental groups of rats which underwent 2 hours of MCA occlusion (each group: n = 5), the largest blood-brain barrier openings and increases in K_i took place in the right neocortical tissue supplied by the occluded right MCA (Table 1). When the 30 min tracer circulation period began 1.5 – 2 min after reperfusion, there was evidence of an early increase in mean K_i to greater than 10-fold of the baseline value. This was followed by a partial recovery, with subsequent K_i measurements and 1 and 4 hours post-reperfusion, which were significantly lower than the acute (1.5 – 2 min) group values, although still elevated above baseline and above values for the contralateral, non-ischemic side. A late opening in the BBB was then demonstrated 4 and 22 hours post-reperfusion. This was most pronounced between 22 and 46 hours (Figure 1).

Striatum removed from the right hemisphere of stroked rats exhibited a slight but significant elevation in K_i , 1.3 – 2 min post-reperfusion (Table 1). This change was no longer present at 1 or 4 hours post-reperfusion, however, a significant increase in K_i was demonstrated between 22 and 46 hours after reperfusion. The dorsal hippocampus ipsilateral to the MCA occlusion showed little change in K_i , except at the 46 hour time point, when a slight elevation was present. In the contralateral, non-ischemic

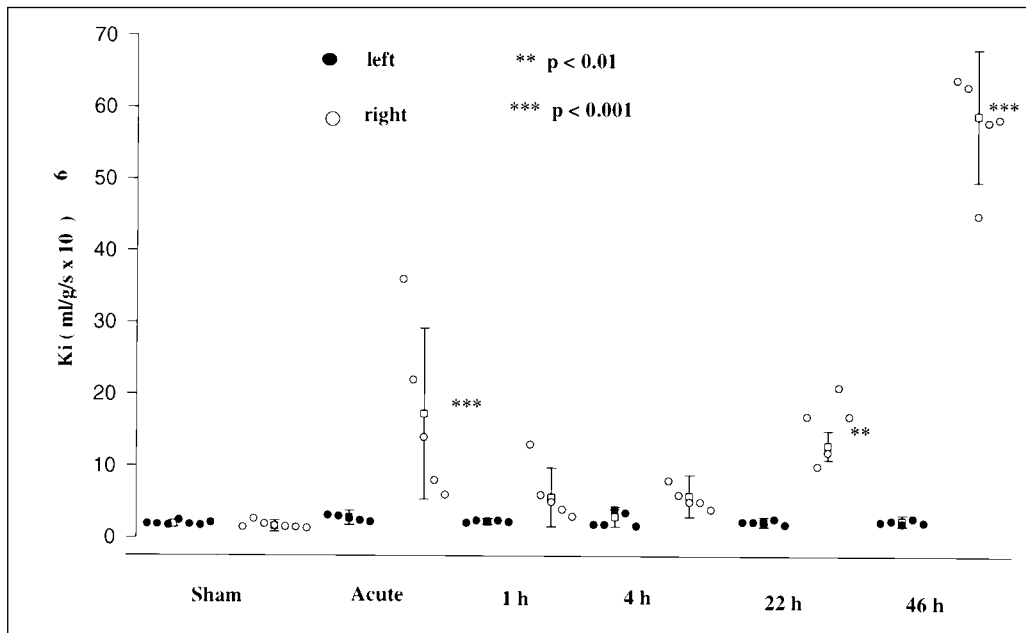


Figure 1: Biphasic opening of the blood-brain barrier in right cerebral cortex of the rat after 2 hours of occlusion and 46 hours of reperfusion of the right MCA. Opening of BBB indicated by increased transfer constant K_i for blood-to-brain diffusion of ^3H -sucrose. The K_i values for the right cortex (ipsilateral to the MCA occlusion) and left cortex from individual animals are represented by open and shaded circles, respectively. The open square with error bars represent the mean \pm SD for each time period. ** $p < 0.01$; *** $p < 0.001$, significantly elevated from control values.

hemisphere, all regions exhibited mean K_i values slightly higher than the baseline, sham stroke values. However, in no instance was this statistically significant.

Experiment 2

Table 2 shows the effect of mild or moderate hypothermia for 6 hours post-reperfusion on BBB opening caused by MCAO. Mean K_i measured 46 hours post-reperfusion was lower in rats that underwent hypothermia (32°C - 33°C) compared to that of normothermic controls. With moderate hypothermia of 28°C - 29°C, the reduction in BBB opening was more striking.

Experiment 3

Table 3 reports edema measurements based on the wet to dry weight difference as a percentage of water per cerebral hemisphere ($\text{mL.g}^{-1} \times 100$). In 5 rats sacrificed 4 hours post-reperfusion, the values (mean \pm SD) were $80.4 \pm 0.3\%$ and $78.5 \pm 0.2\%$ for the right and left sides, respectively ($p < 0.001$). Furthermore, the mean % H_2O measured in the stroked hemisphere at 22 hours post-reperfusion was significantly higher than that at 4 hours ($p < 0.01$), whereas, in the non-ischemic hemisphere there was no significant difference between these two time points.

Table 2: Effect of Mild and Moderate Post-Ischemic Hypothermia on Transfer Constant (K_i) (Experiment 2)

Variability Group (n)	Right (Mean \pm SD) ($\text{mL.g}^{-1}.\text{s}^{-1} \times 10^6$)			Left (Mean \pm SD) ($\text{mL.g}^{-1}.\text{s}^{-1} \times 10^6$)		
	Cortex	Striatum	Hippocampus	Cortex	Striatum	Hippocampus
Normothermia (8)	30.6 \pm 9.1	5.2 \pm 3.0	2.0 \pm 0.5	1.2 \pm 0.2	1.1 \pm 0.3	1.5 \pm 0.3
Hypothermia (7) 32-33°C	23.1 \pm 9.1	4.0 \pm 1.5	1.9 \pm 0.7	1.3 \pm 0.4	1.1 \pm 0.5	1.6 \pm 0.7
Normothermia (7)	34.7 \pm 10.9	5.0 \pm 1.3	2.7 \pm 1.2	2.0 \pm 0.4	1.0 \pm 1.0	2.3 \pm 1.0
Hypothermia (7) 28-29°C	15.4 \pm 8.8**	3.7 \pm 1.7	1.8 \pm 0.7	1.5 \pm 0.2	1.2 \pm 0.3	1.8 \pm 0.4

** $p < 0.01$, student t-test.

Table 3: Percentage of Water in MCA Model (Experiment 3)

Group (n)	Variability	Right Cortex % of Water (Mean ± SD)	Left Cortex % of Water (Mean ± SD)
Normothermia			
4 hours post-reperfusion (5)		80.4 ± 0.3 ^a	78.5 ± 0.2
22 hours post-reperfusion (5)		83.7 ± 0.6 ^{ab}	78.8 ± 0.1
46 hours post-reperfusion (6)		86.1 ± 0.7 ^{ab}	79.8 ± 0.7
Moderate Hypothermia (6)		85.7 ± 1.4 ^{ab}	79.6 ± 0.3

p < 0.01 for ANOVA plus Tukey's test.
a – right side values different from corresponding left side values; b – different from 4 hr post-reperfusion value.

Post-ischemic hypothermia (28°C - 29°C) for the first 6 hours had no ameliorative effect upon the edema measured in the cortex 46 hours after reperfusion. The mean (± SD) percentage water (ml.g⁻¹ × 100) for the normothermic group was 86.1 ± 0.7% for the post-ischemic right cortex and 79.8 ± 0.7% for the contralateral left cortex, versus 85.7 ± 1.4% (right) and 79.6 ± 0.3% (left) for that of the hypothermic group.

Experiment 4

Despite the failure of moderate post-ischemic hypothermia to reduce the accumulation of edema in hypothermia (28°C - 29°C) for the first 6 hours of the 46 hour reperfusion period, this resulted in a mean cortical infarct volume of 122 ± 57 mm³ (n = 6) (Figure 2), which was significantly lower than that of the normothermic control group (175 ± 22 mm³, n = 6) (p < 0.05) (Table 4). (Table 4). Animals receiving 6 hours of mild hypothermic reperfusion (32°C - 33°C) had a total infarct volume of 149 ± 39 mm³ (n = 6) at 46 hours, which was less than that of the control group, but not significantly so. The reduction in the size of injury relates to smaller volumes of infarction rather than differences in the amount of swelling or edema, confirming Experiment 3.

DISCUSSION

In these transient ischemic experiments, the widest BBB openings and increases in K_i were seen in the post-ischemic right cerebral cortex, which was dissected to include both the core and edge of the region perfused by the MCA. The opening was clearly biphasic, characterized by an initial 10-fold augmentation in K_i during the first half hour of reperfusion, followed by partial closing, and then a delayed, but progressive, opening between 22 and 46 hours post-reperfusion. Moderate hypothermia during ischemia dramatically reduced infarction and edema, as well as preventing BBB opening, but also had partial effects on infarct size when instituted during the post-ischemic period following normothermic ischemia.¹¹ In these studies, we have demonstrated that postischemic moderate hypothermia affects not only the size of the infarct, it does so in tandem with reductions in the opening of the BBB, not by simply reducing the amount of vasogenic edema, but possibly by interfering with the post-ischemic inflammatory response.

This profile of BBB injury shows similarities to findings based

on the assessment of Evan's Blue dye extravasation in the cat.¹³ Following one hour of temporary MCA occlusion, dye injected i.v. early in reperfusion caused staining of brain parenchyma. This was followed by a refractory period, and then a delayed opening, which was visible in cats sacrificed five hours or three days post-stroke. The initial acute opening has been described as a 'hemodynamic' BBB opening.^{4,13} Because of acidosis, loss of autoregulation, and vasodilation of the cerebral vasculature, reperfusion results in excessive blood flow or 'luxury perfusion'. High intraluminal blood pressure in the cerebral microvasculature has been shown to induce abnormal pinocytotic transport across endothelial cells, and opening of interendothelial tight junctions.^{14,15} A significant role of arterial pressure in the degree of post-reperfusion opening after

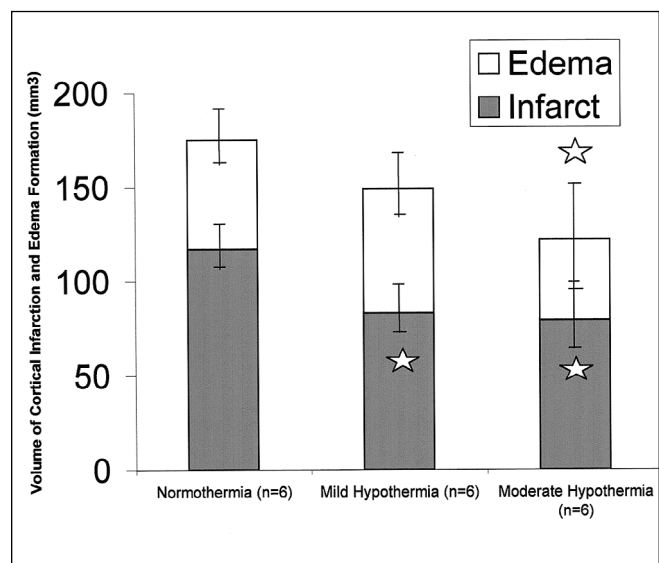


Figure 2: Volume of neocortical infarction for each rat following 2 hours of normothermic transient MCA occlusion and 46 hours of reperfusion, in the first 6 hours of which animals were maintained at either 37°C-38°C (normothermia), 32°C-33°C (mild hypothermia), or 28°C-29°C (moderate hypothermia). The mean edema and infarct sizes are displayed ±SD (the asterisk denotes significantly less injury (p < 0.05). The n value for each group is indicated in brackets.

Table 4 : Effects of infarct volume and edema formation following post-ischemic hypothermia in MCA Model (Experiment 4)

Group (n)	Variability	Edema (mm ³) (Mean ± SD)	Infarction (mm ³) (Mean ± SD)	Total Infarct Volume (mm ³) (Mean ± SD)
Normothermia (6)		58 ± 17	117 ± 13	175 ± 23
Mild Hypothermia (6)		67 ± 23	83 ± 31*	149 ± 39
Moderate Hypothermia (6)		43 ± 26	79 ± 33*	122 ± 57

* p < 0.05, between treatments for ANOVA test.

MCAO has been demonstrated.¹⁶ After 3 hours of MCAO and 30 min re-circulation, BBB opening to Evan's Blue dye was greatly augmented in rats that had been rendered hypertensive with phenylephrine during the reperfusion period. The magnitude of acute hyperemic BBB opening, and of modulating factors, such as blood pressure, would appear to be an important consideration in utilizing and interpreting the MCAO model. For example, factors such as hypertension, which augment post-reperfusion opening, and the ensuing homeostatic changes might thereby indirectly influence subsequent neuropathological events. On the other hand, one might anticipate that the degree of early post-ischemic BBB opening could have a positive impact on the efficacy of experimental drugs when the chemical nature of these compounds limits their ability to cross the normal BBB. For instance, with the competitive AMPA antagonist NBQX, which penetrates the blood-brain barrier, effective concentrations of the drug are achieved following transient focal ischemia, perhaps as a consequence of the opening of the barrier at the time the drug is circulating.¹⁷

In this study, partial recovery from the acute opening of the BBB was evidenced by the fact that K_i values at one or four hours post-reperfusion were significantly lower than those measured acutely, but were higher than baseline values, or values for contralateral non-ischemic cortex. The second part of the biphasic opening was then indicated by the significant elevation of K_i at 22 hours post-reperfusion as compared to that at four hours, with an even more dramatic upward increment taking place between 22 and 46 hours. Accounting for this time delay would seem important in any proposal on the cause(s) of BBB opening, which presumably differs from that underlying the acute post-ischemic opening. In fact, the delayed BBB opening to ³H-sucrose is consistent with the published observations that between 24 and 48 hours after transient MCAO, there occurs a rapid evolution of delayed edema, which peaks within this time period, and that infiltration of polymorphonuclear cells follows a similar time course.¹⁸ Vascular endothelial leakiness was proposed to result from the release of lipid inflammatory mediators through the interaction of injured tissue with infiltrating leukocytes and aggregating platelets.¹⁹ Among the possible mediators of BBB dysfunction and formation of vasogenic edema, proteases, bradykinin, histamine, and eicosanoid products of arachidonic acid metabolism and free radicals have been strongly implicated.²⁰

It is well documented that neuronal injury is reduced by induction of hypothermia during ischemia, or even by its

induction during the post-ischemic reperfusion period.²¹ The experiments in the present study quantitate for the first time a protective effect of post-ischemic hypothermia on the delayed BBB injury that follows temporary MCAO. Mean K_i values after 46 hours of reperfusion were more than 50% lower in rats that underwent cooling to 29°C for 6 hours. Although a light degree of halothane anesthesia in the cooled rats may have contributed a protective effect, efficacy of lowered brain temperature per se was suggested by the fact that 28°C - 29°C was more protective than 32°C - 33°C. Separate experiments showed, however, that the 6 hours of hypothermic treatment at 28°C - 29°C did not reduce the amount of edema present at 46 hours post-reperfusion, even though this treatment appeared to favourably affect both BBB damage and infarct volume.

Post-ischemic hypothermic protective mechanisms may be related to a slight attenuation in the reperfusion hyperemia. During reperfusion, hypothermia may reduce leukotrienes,²² improve glucose utilization and blood flow,²³ and slow free radical reactions and the propagation of lipid peroxidation cascades. This could prevent the leakage of proteins and the accumulation of extracellular fluid²⁴ and inhibit the biosynthesis, release and uptake of neurotransmitters, such as glutamate and dopamine.²⁵ There are two massive glutamate release points in the first 4 hour period of reperfusion following MCA/CCA occlusion.²⁶ The quantal release of glutamate has been recently correlated with the size of neocortical infarction in focal ischemia²⁷ and may result in endothelial cell damage in the BBB. The blockade on the non-NMDA glutamate receptors attenuates brain damage.¹⁷ Therefore, any reduction of glutamate concentration in the extracellular space by delayed hypothermia may protect against brain damage.

In conclusion, following transient focal ischemia, measurements of K_i for BBB permeation of ³H-sucrose have demonstrated both an acute opening, likely hemodynamic in nature, and a delayed opening of the ipsilateral cortex MCAO. Clearly, hypothermia has potent effects on BBB opening²⁸ and does reduce infarct size, but through mechanisms other than reducing edema. The quantitative power of K_i measurements should facilitate the exploration of drug or other treatments to offset the disadvantages of reperfusion therapy (BBB opening and edema) and facilitate its benefits. The understanding of the interplay between microvascular damage, edema, inflammation, and neuronal death hours after thromboembolic stroke is critical to the development of successful stroke therapies.

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