Effect of Cerebral Perfusion Pressure on Cerebral Cortical Microvascular Shunting at High Intracranial Pressure in Rats

Denis E. Bragin, PhD; Rachel C. Bush, BA; Edwin M. Nemoto, PhD

Background and Purpose—Recently, we showed that decreasing cerebral perfusion pressure (CPP) from 70 mm Hg to 50 mm Hg and 30 mm Hg by increasing intracranial pressure (ICP) with a fluid reservoir induces a transition from capillary (CAP) to microvascular shunt (MVS) flow in the uninjured rat brain. This transition was associated with tissue hypoxia, increased blood–brain barrier (BBB) permeability, and brain edema. Our aim was to determine whether an increase in CPP would attenuate the transition to MVS flow at high ICP.

Methods—Rats were subjected to progressive, step-wise increases in ICP of up to 60 mm Hg by an artificial cerebrospinal fluid reservoir connected to the cisterna magna. CPP was maintained at 50, 60, 70, or 80 mm Hg by intravenous dopamine infusion. Microvascular red blood cell flow velocity, BBB integrity (fluorescein dye extravasation), and tissue oxygenation (nicotinamide adenine dinucleotide) were measured by in vivo 2-photon laser scanning microscopy. Doppler cortical flux, rectal and cranial temperatures, ICP, arterial blood pressure, and gases were monitored.

Results—The CAP/MVS ratio increased (P<0.05) at higher ICP as CPP was increased from 50 to 80 mm Hg. At an ICP of 30 mm Hg and CPP of 50 mm Hg, the CAP/MVS ratio was 0.6±0.1. At CPP of 60, 70, and 80 mm Hg, the ratio increased to 0.9±0.1, 1.4±0.1, and 1.9±0.1, respectively (mean±SEM; P<0.05). BBB opening and increase of reduced form of nicotinamide adenine dinucleotide occurred at higher ICP as CPP was increased.

Conclusions—Increasing CPP at high ICP attenuates the transition from CAP to MVS flow, development of tissue hypoxia, and increased BBB permeability. (Stroke. 2013;44:177-181.)

Key Words: blood–brain barrier ■ cerebral blood flow ■ cerebral perfusion pressure ■ hypoxia ■ intracranial pressure ■ microvascular shunts

Methods

Animals and Surgical Procedures

Protocol #100916 was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of New Mexico Health Sciences Center. Acclimated male Sprague-Dawley rats were used (300–350 g, n=28; Harlan Laboratories, Indianapolis, IN). Anesthesia was induced with 4% isoflurane/70% nitrous oxide and 30% oxygen. The rats were intubated with a 1.4-G × 1.9-inch catheter and mechanically ventilated (Harvard Apparatus) on 2% isoflurane/30% oxygen/70% nitrous oxide with tidal volume of 2.0 to 2.5 mL at a rate of 55 to 65 per minute. Rectal temperature was kept at 37±0.5°C by a heated water blanket. Atropine (0.2 mg intraperitoneally) was used to reduce mucous secretions. Femoral artery catheters (PE-50) were used to monitor arterial blood pressure and blood sampling (0.3 mL each). Double-lumen femoral vein catheters (Braintree Scientific) were used for dopamine infusion, fluid replacement (lactated Ringers, 1 mL/hour), and fluorescein-dextran injection. The rats were placed in a stereotaxic head frame (Kopf Instruments). A catheter (PE-50) was inserted into the cisterna magna and glued in place to monitor and manipulate ICP by a reservoir of artificial cerebrospinal fluid. A craniotomy (5 mm diameter) over the left parietal cortex was filled with 1.5% agarose in saline and a cover glass slip over the craniotomy was glued to the skull. A cranial temperature probe was used to monitor brain temperature.

Experimental Paradigm

The animals were studied in 4 groups (n=7). CPP was maintained at 50 mm Hg, 60 mm Hg, 70 mm Hg, and 80 mm Hg (Table). In each group, ICP was sequentially increased from 10 to 30, 40, and 60 mm Hg by raising the artificial cerebrospinal fluid reservoir while CPP was manipulated by titrated intravenous dopamine (1 mg/mL) infusion with a Syringe Pump (Harvard Apparatus). The dopamine dose infused ranged from 67±16 to 400±70 μg/kg per minute (mean±SEM). The average time to achieve a stable elevation in CPP was 7.4±5.2 min. Thirty minutes at each CPP was sufficient for stabilization of physiological variables and completion of the measurements. Dopamine was chosen as the vasopressor because norepinephrine and phenylephrine induced severe arterial acidosis that was difficult to control with intravenous sodium bicarbonate. Arterial acidosis with dopamine was minimal and easily controlled by intravenous sodium bicarbonate. Arterial acidosis and phenylephrine induced severe arterial acidosis that was difficult to control with intravenous sodium bicarbonate.

In Vivo 2-Photon Laser Scanning Microscopy

NADH autofluorescence was measured using 2-photon laser scanning microscopy as described. Fluorescence was emitted by 740-nm center wavelength and filtered at 425 to 475 nm.² Selective planar scans of fluorescence intensity were obtained in 10-μm steps starting at 100 μm from the pia mater at each ICP. In offline analyses, average intensity was calculated from the maximal intensity projection for each ICP.

Cortical Doppler Flux

Relative changes in cortical flow were measured continuously by Doppler flux using a single-fiber 0.8-mm-diameter surface Doppler probe (Moor Instruments) on the temporal bone (burr hole) below the optical window.

Statistical Analysis

Statistical analyses were performed by independent Student t test or Kolmogorov-Smirnov test when appropriate. Differences between groups were determined using 2-way ANOVA for multiple comparisons and post hoc testing using the Mann–Whitney U test. Bonferroni multiple-comparison tests was used for post hoc analysis when the effects of different CPPs were compared against each other. Significance level was preset to P<0.05. Data are presented as mean±SEM.

Results

Physiological variables were within normal limits throughout the studies and were not significantly different (Supplementary Table I). However, blood glucose levels were elevated in all groups, likely as a result of the stress of surgery and anesthesia. Variations in blood gases were adjusted by manipulation of the rate and volume of the ventilator. Base deficits less than −5.0 mEq/L were corrected by slow intravenous injection of 8.4% sodium bicarbonate.

Table. Experimental Paradigm for 4 Study Groups

<table>
<thead>
<tr>
<th>Time From the Start, min</th>
<th>Group I (CPP=50 mm Hg)</th>
<th>Group II (CPP=60 mm Hg)</th>
<th>Group III (CPP=70 mm Hg)</th>
<th>Group IV (CPP=80 mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ICP (51.4±3.3)</td>
<td>ICP (62.2±3.5)</td>
<td>ICP (70.6±4.4)</td>
<td>ICP (83.4±5.1)</td>
</tr>
<tr>
<td>0</td>
<td>10.6±2.3</td>
<td>10.8±4.1</td>
<td>11.2±3.4</td>
<td>10.3±3.5</td>
</tr>
<tr>
<td>30</td>
<td>29.8±4.1</td>
<td>30.5±3.6</td>
<td>31.6±4.1</td>
<td>30.2±4.8</td>
</tr>
<tr>
<td>60</td>
<td>40.1±5.2</td>
<td>42.6±5.3</td>
<td>40.4±4.8</td>
<td>41.6±6.1</td>
</tr>
<tr>
<td>90</td>
<td>60.3±4.4</td>
<td>61.3±5.6</td>
<td>63.1±6.7</td>
<td>60.5±7.2</td>
</tr>
</tbody>
</table>
RBC Flow Velocities

Microvascular blood flow was measured by fluorescein-dextran labeling of plasma to observe RBCs as negatively stained stripes in a background of labeled plasma (Figure 1A). Line scans were performed at each ICP on ≈100 microvessels (3–15 μm diameter) from the regions imaged by 2-photon laser scanning microscopy at several depths (100–300 μm) from the pia mater. A line scan through a microvessel leads to a sequence of alternating bright and dark pixels corresponding to labeled plasma and unlabeled RBCs. The result is diagonal bands in a space–time image as illustrated in Figure 1B. The slope of the stripes inversely reflects RBC velocity.

As we previously reported,5 at a normal CPP of 70 mmHg the proportion of low-velocity capillary (<1 mm/sec, 3–7 μm diameter) was 67.2%, which is significantly greater than the 32.8% of high-velocity microvessels (>1 mm/sec, 8–15 μm diameter). CPP reduction to 30 mmHg decreased the proportion of low-flow microvessels to 48.8% and increased the proportion of high-velocity microvessels to 51.2%. This redistribution was associated with hypoxia, brain edema, and BBB leakage, which is a hallmark of non-nutritive MVS flow.

The capillary/microvascular shunt (MVS) flow ratio we use here (Supplementary Figure I) reflects the relative proportion of capillary to MVS flow (Figure 1C). In all groups at normal ICP of 10 mmHg, the capillary/MVS ratio was similar to control, with an average value of 2.15±0.29 for all 24 animals. A progressive increase in ICP from 10 mmHg to 30, 40, and 60 mmHg at constant CPPs resulted in a progressive increase in the number of microvessels, with flow velocities >1.0 mm/s suggesting a shift from low-velocity capillary to high-velocity MVS flow. At ICP ranging from 30, 40, and 60 mmHg, and CPP at 50, 60, 70, and 80 mmHg, the capillary/MVS ratio increased significantly, indicating a reduction in microvascular shunting (Supplementary Table III). Thus, increasing CPP at a given ICP attenuated the transition from capillary to MVS flow.

The increase in Doppler flux with step-wise increase in ICP at a constant maintained CPP reflects the flow shift from low-velocity capillary to high-velocity MVS flow (Supplementary Table III). The increase in Doppler flux with ICP elevation was steeper at lower CPP than at higher CPP, correlating with a higher proportion of MVS flow at any given increased ICP and lower CPP.

Tissue NADH

NADH is an indicator of the status of mitochondrial oxidation. NADH is fluorescent, whereas oxidized NAD+ is not. Thus, increased NADH autofluorescence reflects tissue hypoxia.21,22 At ICP of 10 mmHg and at CPP of 70 mmHg, NADH autofluorescence was evenly distributed in a rat parietal cortex (Figure 2A). NADH fluorescence in a tissue more proximal to microvessels was less bright than distal, reflecting better oxygenation resulting from oxygen gradients.21 In controls, NADH autofluorescence was unchanged over 120 min. Progressively increasing ICP resulted in a marked increase in NADH fluorescence, suggesting tissue hypoxia in all 4 experimental groups (Figure 2B and 2C and Supplemental Table IV; P<0.05 and P<0.01). The increase in NADH was less at higher CPP.

BBB Permeability

In intact brain, bright vessels filled with fluorescein-dextran were clearly seen over the dark background of unstained tissue (Figure 3A). Increased BBB permeability leaks fluorescein-dextran out of microvessels into tissue (Figure 3B). The table in Figure 3C demonstrates the number of rats out of 7 for each group showing dye extravasation. In a control group (ICP 10
fuse the infracted tissue without nutrient and gas exchange.23,24 whereas larger thoroughfare channels shunts continue to per-
capillary rarefaction in infarcted tissue observed histologically,
lary beds. These distinctions are supported by observations of
range in diameter from 5 to 12
mm, the incidence of BBB leakage decreased. Average dye fluorescence in each group showed a significant
gradual increase of fluorescence in brain tissue, with ICP increasing from 10 to 60 mm Hg, reflecting opening of the BBB
(P<0.05, P<0.01). Higher CPPs attenuated BBB degradation compared with lower CPPs (Figure 3D).

Discussion

Important distinctions are to be made regarding the cerebral microvasculature. Capillaries are distinguished from shunts by
their size (3–7 μm diameter), branching, and tortuosity.5–9,11,19 Larger-diameter microvessels from 8 μm to 45 μm are in the
range of arterioles and venules and arterio-venous, arterio-arte-
tio, and veno-veno shunts that are precapillary shunts capable of shunting blood away from capillary beds.6–9,11 Thoroughfare
channels as described by Hasegawa and Ravens,6,11 however, range in diameter from 5 to 12 μm and course through capil-
ary beds. These distinctions are supported by observations of
capillary rarefaction in infarcted tissue observed histologically,
whereas larger thoroughfare channels shunts continue to per-
fuse the infracted tissue without nutrient and gas exchange.23,24
Thus, whereas arterio-veno, arterio-arterio, and veno-veno shunts would result in capillary rarefaction, persistence of
thoroughfare channels shunts continues non-nutritive perfu-
sion through the brain. The result is hyperemia through
infarcted tissue without gas or nutrient exchange, resulting in
tissue hypoxia and increased BBB permeability. A PO2 gap is
noted with low tissue PO2 and high cerebral venous PO2.25 The
percentage of these shunts in the brain is small relative to capil-
laries, but exactly what percentage they represent is unknown
and never has been quantitated.19 Our data in the normal rat
brain suggest a value of ≈35%, however, representing not only
thoroughfare channels shunts but also all microvessels includ-
ing arterioles and venules in the range of 8 to 15 μm diameter.

Increased BBB permeability as a result of the increase in capi-
illary pressure also would lead to the initiation of inflammatory
mediators such as cytokines and tumor necrosis factor-α from
endothelial cells, pericytes, mast cells, and neurons, and from
the migration of neutrophils, macrophages, and microglia into
the brain parenchyma.26,27 However, the specific sequence of events in these inflammatory processes requires further investigation.

The use of dopamine to increase CPP may affect MVS flow not only by the increase in CPP but also through vasoactive effects.
Dopamine has both β2-adrenergic chronotropic and inotropic effects on the heart and also β2-adrenergic vasodilatory and α1-
adrenergic vasoconstrictive effects on cerebral blood vessels.28,29
At low doses, dopamine induces a vasoconstrictive α1-adrenergic
effect, whereas at higher doses it induces a vasodilatory β2-
adrenergic vasodilatory effect. In unanesthetized monkeys, we
reported a 20% to 30% increase in CBF and cerebral metabolic
rate for oxygen with dopamine infusion at 100 μg/kg per minute.30
The extent to which these effects of dopamine on the cerebrovas-
culature affected MVS flow remains to be determined.

This study is our first attempt to use the transition from capi-
LLARY to MVS flow to study the interaction of CPP and ICP in the
uninjured brain. The results show that increasing CPP at
high ICP from 10 to 60 mm Hg progressively attenuates the
transition from capillary to MVS flow. The range of ICP studied
is higher than that tolerated clinically but shows that at
high ICP, the effects of increasing CPP on the capillary/MVS
ratio is continuous and monophasic.

Determination of an optimal CPP in patients with elevated
ICP is complex. It is multifactorial in causation and varies with
magnitude of injury severity and the increase in edema and
cerebral blood volume. This study is an extension of previous
studies2–4 showing that increased ICP results in a decrease in
the critical CPP of CBF autoregulation. The decrease in critical
CPP was attributable to the appearance of apparently preserved
autoregulation caused by a transition to pathological MVS flow
and a falsely elevated CBF and autoregulation.5 This phenom-
emon in the normal brain is important because of the dynamics
of the susceptibility of normal brain to increased ICP, which
also coexists in the injured brain and provides a basis for com-
parison with future studies in the traumatized or injured brain.

CBF autoregulation has been historically evaluated by the
relationship between CBF and CPP, but our studies show that
at high ICP the relationship between CBF and CPP can be
misleading. The conventional CBF autoregulation curve for
determination of the critical CPP fails at high ICP as a result
of microvascular shunting. In the injured brain, CBF autoreg-
ulation may be accurately assessed by transient increases in
arterial pressure while recording the change in CBF or ICP. A
change in CBF in response to a change in CPP reflects cere-
brovascular reactivity, and the change in ICP reflects pressure
reactivity.31–33
The transition from capillary to MVS flow secondary to increased ICP likely is caused by an increase in cerebral venous back pressure attributable to the increase in CSF pressure. Increased venous pressure decreases the transcapillary pressure gradient, resulting in decreased capillary flow and arteriolar dilation, thereby increasing the transcapillary pressure gradient to restore capillary flow at a higher capillary hydrostatic pressure. The higher capillary hydrostatic pressure promotes the development of brain edema and increased capillary resistance, which ultimately redirect flow through lower resistance microvascular shunts resulting in capillary rarefaction and non-nutritive hyperemia. Elevated CPP with high ICP should promote flow through high-resistance capillaries and reduce flow through MVS.

In summary, our studies show that increased CPP at high ICP attenuates the transition from capillary to MVS flow. The question remains as to when it is safe to increase CPP. CPP cannot be increased in a patient with high ICP and loss of CBF autoregulation, but patients with high ICP and intact CBF autoregulation may benefit; however, that remains to be determined.

Sources of Funding
This work was supported by National Institutes of Health grants NS061216 and CoBRE8P30GM103400-01, Dedicated Health Research Funds from the University of New Mexico School of Medicine, and American Heart Association grant 12BGIA11730011.

Disclosures
None.

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Stroke. 2013;44:177-181; originally published online November 29, 2012;
doi: 10.1161/STROKEAHA.112.668293

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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