When cerebral perfusion pressure (CPP) is reduced by increasing intracranial pressure (ICP) instead of lowering arterial blood pressure, the critical CPP of cerebral blood flow (CBF) autoregulation decreases from 60 mm Hg to 30 mm Hg. The reason for this decrease in critical CPP was unknown but might be interpreted as improved CBF autoregulation at high ICP. We hypothesized instead that the decrease in critical CPP at high ICP was attributable to a pathologically maintained CBF caused by microvascular shunting (MVS). We tested this hypothesis by decreasing CPP in rats by either increasing ICP or decreasing arterial pressure stepwise from 70 mm Hg to 50 mm Hg and 30 mm Hg while measuring microvascular flow in capillaries (3–7 μm diameter), microvascular shunts, or thoroughfare channels (8–15 μm diameter). Nicotinamide adenine dinucleotide (NADH) for tissue hypoxia, fluorescein dye extravasation for blood–brain barrier (BBB) permeability, and brain water content were also measured. Decreasing CPP by increasing ICP caused a transition from capillary to MVS flow that did not occur when CPP was decreased by lowering arterial pressure. This transition was clearly associated with the development of tissue hypoxia, brain edema, and increased BBB permeability, which are hallmarks of nonnutritive shunt flow.

In the clinical management of patients with high ICP (>20 mm Hg), the optimal CPP remains a matter of dispute. Nevertheless, recommendations are CPP values between 50 and 60 mm Hg and even up to 70 mm Hg without excessive use of hypertensive agents or fluids. There are 2 schools of thought on the management of CPP in patients with high ICP. One is a CPP-directed approach, suggesting maintenance of CPP up to 80 mm Hg and even higher. The other is an ICP-directed therapy using pharmacologic agents while maintaining CPP between 50 and 60 mm Hg. Our aim in this study was to evaluate the transition from capillary to MVS flow at high ICP as a function of CPP.
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 14-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. 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 was likely as a result of the stress of surgery and anesthesia. Variations in arterial pressure, ICP, and CBF were continuously monitored. Arterial pressure, ICP, and MAP were continuously recorded using Biopac preamplifiers and software. Arterial pressure, ICP, and CBF were 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.

Results

Physiological variables were within normal limits throughout the studies and were not significantly different (Supplementary Table II). 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.

In Vivo 2-Photon Laser Scanning Microscopy of NADH

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. Twenty planar scans of fluorescence intensity were obtained in 10-µm steps starting at 100 µm from the pia matter at each CPP. 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 test 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.

Table. Experimental Paradigm for 4 Study Groups

<table>
<thead>
<tr>
<th>Time From the Start, min</th>
<th>Group I CPP=50 mm Hg (51.4±3.3)</th>
<th>Group II CPP=60 mm Hg (62.2±3.5)</th>
<th>Group III CPP=70 mm Hg (70.6±4.4)</th>
<th>Group IV CPP=80 mm Hg (83.4±5.1)</th>
</tr>
</thead>
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<tr>
<td></td>
<td>ICP</td>
<td>ICP</td>
<td>ICP</td>
<td>ICP</td>
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<tr>
<td>0</td>
<td>10.6±2.3</td>
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<td>10.8±4.1</td>
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<td>11.2±3.4</td>
<td>92.5±5.1</td>
<td>10.3±5.3</td>
<td>92.7±7.6</td>
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<td>30</td>
<td>29.8±4.1</td>
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<td>129.7±13.4</td>
<td>60.5±7.2</td>
<td>139.8±12.8</td>
</tr>
</tbody>
</table>

CPP indicates cerebral perfusion pressure; ICP, intracranial pressure; and MAP, mean arterial pressure.

n=7 rats/group, all measurements in mm Hg, mean±SEM.
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 mm Hg 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 mm Hg 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 mm Hg, 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 mm Hg to 30, 40, and 60 mm Hg 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 mm Hg, and CPP at 50, 60, 70, and 80 mm Hg, 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 mm Hg and at CPP of 70 mm Hg, 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
mm Hg, CPP 70 mm Hg; n=7), only 1 rat showed BBB leakage after 90 minutes of recording (not shown). In groups 1 through 4 with CPP maintained at 50, 60, 70, and 80 mm Hg and with ICP of 10 to 60 mm Hg, 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-arterio, 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 capillary beds. These distinctions are supported by observations of capillary rarefaction in injured tissue observed histologically, whereas larger thoroughfare channels shunts continue to perfuse the injured tissue without nutrient and gas exchange.5,24

Thus, whereas arterio-veno, arterio-arterio, and veno-veno shunts would result in capillary rarefaction, persistence of thoroughfare channels shunts continues non-nutritive perfusion through the brain. The result is marked hyperemia through injured 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 capillaries, 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 including arterioles and venules in the range of 8 to 15 μm diameter.

Increased BBB permeability as a result of the increase in capillary 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.25,27 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 vasconstrictive effects on cerebral blood vessels.28,29 At low doses, dopamine induces a vasconstrictive 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 cerebrovascular affected MVS flow remains to be determined.

This study is our first attempt to use the transition from capillary 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 studies 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. This phenomenon 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 comparison 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 autoregulation 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 cerebrovascular 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.

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Disclosures
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