Functional, Morphological, and Metabolic Abnormalities of the Cerebral Microcirculation after Concussive Brain Injury in Cats

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SUMMARY We induced experimental concussive brain injury by a fluid percussion device in anesthetized cats equipped with a cranial window for the observation of the pial microcirculation of the parietal cortex. Brain injury resulted in transient but pronounced increases in arterial blood pressure and in sustained arteriolar vasodilation associated with reduced or absent responsiveness to the vasoconstrictor effect of arterial hypocapnia and with reduced or absent ability of the vessels to undergo autoregulatory vasodilation in response to reductions in arterial blood pressure. Such vessels had reduced resting oxygen consumption in vitro. Electron microscopic examination of the same vessels that were studied physiologically disclosed the presence of discrete endothelial lesions consisting of either vacuolization or crater formation. Occasionally there was extensive destruction and necrosis of the endothelial cells. There was little or no morphological evidence of vascular smooth muscle damage. There was a close association between the presence of endothelial lesions and vessel dilation and unresponsiveness, suggesting a causal relationship. In cats in which the transient post-traumatic hypertensive episode was prevented, the vessels retained their normal caliber, remained normally responsive, and had no endothelial lesions. The results show that the vascular lesions in the pial microcirculation following this type of brain injury are due to the rise in arterial pressure.


The existence of abnormalities in the cerebral circulation following head trauma has been demonstrated convincingly (Langfitt et al., 1966). Changes in cerebral blood flow (Brown and Brown, 1954; Denny-Brown and Russell, 1941; Overgaard and Tweed, 1974; Taylor and Bell, 1966; Meyer et al., 1970; Hoyer et al., 1972; Fieschi et al., 1972; Bruce et al., 1973; Nilsson, 1976), altered responsiveness of the cerebral circulation to changes in blood pressure (Overgaard and Tweed, 1974; Fieschi et al., 1972; Bruce et al., 1973) and to carbon dioxide tension (Overgaard and Tweed, 1974; Fieschi et al., 1972), increases in intracranial pressure (Bruce et al., 1973), and alterations in vessel caliber (Smith et al., 1969; McCullough et al., 1971; Ommaya et al., 1964; Ekelund et al., 1974) have been described following head trauma in patients or in experimental animals. These changes in the cerebral circulation may contribute importantly to the dysfunction of the injured brain and, in some cases, may actually be the primary cause of such dysfunction. Published studies of the cerebral circulatory disturbances following brain injury have been concerned mainly with alterations in cerebral blood flow in patients with brain injuries and in animals with experimental head injury. Another approach used frequently has been the angiographic study of the large surface vessels following head injury (McCullough et al., 1971; Ekelund et al., 1974; Ommaya et al., 1964). Despite the recognized importance of the microcirculation in the physiological regulation of blood flow, there is a surprising dearth of direct observations of the small surface vessels of the brain following brain injury.

We report here systematic observations of the changes in the pial microcirculation of the anesthetized cat following experimental brain injury induced by fluid percussion.

Methods

Experiments were carried out on cats weighing between 2 and 4.5 kg and anesthetized with sodium pentobarbital (30 mg/kg, iv). After completion of tracheostomy, each cat was ventilated with a positive pressure respirator and received decamethonide (4 mg/kg, iv, for skeletal muscle paralysis). The end-expiratory CO₂ concentration was monitored with a Beckman infrared CO₂ analyzer and was maintained at a constant level between 30 and 35 mm Hg throughout each experiment by adjustment of the respiratory rate and...
volume. Arterial blood pressure was measured with a Statham P23Db pressure transducer connected to a cannula introduced into the aorta through the femoral artery. Arterial blood samples were collected periodically for the determination of $P_{aO_2}$, $P_{aCO_2}$, pH, and hematocrit. Blood gases and pH were determined with Radiometer electrodes. Mean ± standard error for resting blood gases and pH for all cats studied were as follows: $P_{aO_2}$ 110 ± 1.5 mm Hg; $P_{aCO_2}$ 32 ± 0.6 mm Hg; and pH, 7.35 ± 0.01. Hematocrit was measured with a micromethod. Cats with hematocrits less than 30% were not used for these experiments.

With the cat’s head securely positioned in a stereotaxic apparatus, two openings were made in the skull, one for the implantation of a cranial window and the other for the implantation of a right-angle metal shaft which is later connected to the fluid-percussion apparatus. The window was implanted in the left parietal bone just caudal to the coronal suture to overlie the suprasylvian and ectosylvian gyri. These two gyri contain small (<100 μm in diameter) as well as larger vessels (100–400 μm) Kontos et al., 1978) so that arterial vessels over a wide range of vessel size could be observed. The cranial window and the technique for implanting it have been described in detail previously (Levasseur et al., 1975). Approximately 1–2 cm from the hole for implanting the cranial window a second opening, 11 mm in diameter, was made in the right parietal bone. The bone was cauterized to control bleeding. The dura was not removed at this site. The opening was connected to a right-angle metal shaft, 9 mm in internal diameter. Two small screws also were placed in the skull, one rostral and the other caudal to the cranial window and metal shaft without penetrating the full thickness of the skull. The cranial window, the two small screws, and the metal shaft were sealed in the skull with dental acrylic. The purpose of the two small screws was to anchor the window more securely and prevent its dislodgment at the time of injury. The metal shaft was connected to a Statham PA 856-100 transducer housing and this was in turn connected to the fluid percussion device. This device originally was described by Lindgren and Rinder (1969) and was modified by Sullivan et al. (1976) for use in the cat. It consists of a Plexiglas cylindrical reservoir 60 cm long, 4.5 cm in diameter and 0.5 cm thick. One end of it was connected to the Statham transducer housing, while the other end was bounded by a Plexiglas piston mounted on O rings. The exposed end of the piston was covered with a sponge rubber pad. The entire system was filled with 0.9% NaCl solution warmed to 37°C. To facilitate removal of the air bubbles from the system, a small amount of sodium taurocholate was added to reduce surface tension. The percussion device was supported securely by two brackets mounted on the metal platform which also supported the cat and the microscope assembly used for the observation of the pial microcirculation. Brain injury was produced by striking the piston with a 4.8-kg steel pendulum. The intensity of the injury was controlled by varying the height from which the pendulum was allowed to fall. The pressure change generated by the device was recorded with a Statham PA 856-100 transducer. It consisted of a pressure pulse of variable amplitude, usually 1 to 4 atm, and of fairly constant duration of 21–24 msec. This pressure pulse was recorded on a Tektronix storage oscilloscope. The amplitude of the pressure pulse was used to quantify the intensity of the injury. The oscilloscope was triggered photoelectrically by a sensor activated by the descent of the pendulum.

The pial microcirculation on the parietal cortex was observed through the cranial window with a Leitz microscope equipped with a Vickers imagesplitting device. The cranial window was filled with artificial cerebrospinal fluid (CSF) having the same composition as normal CSF for cats (Levasseur et al., 1975). The window has three openings, each of which was attached to stopcocks. One of the openings was used for monitoring intracranial pressure with a Statham transducer. The other two could be used as an inlet and outlet for flushing the space under the cranial window with various solutions.

The experimental design was as follows: After implantation procedures had been completed, the cat was placed under the microscope and control observations of blood pressure, intracranial pressure, and arterial vessel diameter were made. In the initial experiments, in which the time course of the vessel caliber changes was not known, only one or two vessels were monitored in each cat. The diameter of each vessel was recorded continuously for the first 5 minutes after brain injury and then for 1 minute every 5 minutes for the succeeding 55 minutes. In subsequent experiments, having established the time course of the diameter and blood pressure changes, we generally made observations in the steady state approximately 30–60 minutes after brain injury. In these experiments, several arterial vessels of various sizes were measured.

The responsiveness of vessels to changes in $CO_2$ was determined by inducing arterial hypocapnia via passive hyperventilation through an increase in the rate and volume of the respirator. The magnitude of the responses to arterial hypocapnia in normal pial arterioles is independent of vessel size, whereas the magnitude of the responses to arterial hypercapnia is size-dependent (Kontos et al., 1979). For this reason it was preferable to use the responses to hypocapnia rather than those to hypercapnia as a measure of the responsiveness of vessels to $CO_2$.

The responses of vessels to alterations in blood pressure were studied by inducing graded hypotension by cannulating the descending aorta with a wide-bore tubing, which was in turn connected to a reservoir filled with heparinized 0.9% NaCl solution and set at the desired height to produce a given change in blood pressure. In these experiments, the
arterial pressure was measured with a Statham strain gauge connected to a cannula introduced into the brachial artery.

After completion of the physiological studies, morphological examination of the pial vessels was conducted in 34 cats by both scanning and transmission electron microscopy. The vessels were harvested by a method that permits identification and study of the same vessels whose functional characteristics were investigated. Perfusion-fixation of the brain was accomplished via cannulas inserted in both the right and left carotid arteries through a Y connection. The chest cavity and the pericardium were opened to allow for right atrial drainage and, subsequently, the carotid arterial lines were opened to a perfusion reservoir placed at a height sufficient to provide a driving pressure of 100 mm Hg. The cerebral vascular bed first was perfused with 0.9% NaCl solution and then with a solution of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M Na-phosphate buffer.

After perfusion, the cranial window was removed and the cranial vault opened. The brain then was removed and placed in fresh fixative for 1 hour. Next the brain was washed for an additional 2 hours in chilled (4°C) Na-phosphate buffer. The pia mater was peeled from the surface of the parietal cortex and postfixed in 1% buffered osmic acid for 1 hour. In a Na-phosphate-buffered bath, the vessels were viewed through an American Optical dissecting microscope and dissected free from the pia mater. With the aid of a map drawn previously and demonstrating the pial vasculature beneath the window, identification of the same vessels that had been studied physiologically was achieved. Once the appropriate pial vessels had been identified, alternate segments of each vessel were prepared for scanning and transmission electron microscopic analysis.

Specimens for scanning electron microscopy were dehydrated in graded ethanol solutions, subjected to critical point drying, and mounted on a circular aluminum stub 1 cm in diameter. The specimens next were coated with gold 100–400 Å thick in a vacuum chamber and examined with a Hitachi S-500 scanning electron microscope. Those adjacent vessel segments used for transmission electron microscopy were dehydrated in graded concentrations of ethanol and propylene oxide, and embedded in Epon 812. Thick sections were cut with glass knives and examined for purposes of orientation. Serial ultra-thin sections then were cut with a diamond knife, picked up on uncoated 200 mesh grids, stained with uranyl acetate and lead citrate, and examined with a Hitachi HS-7 electron microscope. Five cats serving as morphological controls underwent all surgical procedures but were not subjected to mechanical brain injury.

In 15 cats, the vessels that previously had been studied were carefully removed from the surface of the brain by microdissection, and their oxygen consumption rates were measured in vitro in the Cartesian diver microrespirometer. The oxygen consumption rates from three to five segments of each vessel were averaged. The technique for removing the pial arterioles and measuring their oxygen consumption was described in detail previously (Navari et al., 1979). In earlier experiments on normal pial vessels, we found that the oxygen consumption rates of the vessel wall were size-dependent (Navari et al., 1979). For this reason, the results from the present experiments were compared to the oxygen consumption rates of normal vessels of the same in vivo size. Statistical comparisons were carried out by a t-test when two groups of data were compared, or by analysis of variance when more than two groups of data were compared.

Results

There were 13 failures out of 165 cats subjected to brain injury. In five of these, the glass plate of the window broke at the time of the delivery of the blow. All of these five occurred with injury levels in excess of 3 atm. In eight cats there was subarachnoid hemorrhage, sufficiently severe to obscure the brain surface under the window and prevent continued measurement of vessel caliber. In these experiments there were marked elevations of intracranial pressure ranging from 20 to 140 mm Hg. Pathological examination of the brain in these cats revealed extensive subarachnoid hemorrhage originating in the brain stem.

Time Course of Vessel Caliber Changes

The time course of changes in arterial blood pressure, intracranial pressure and pial arteriolar caliber for 3 different intensities of brain injury were studied in five groups of cats. The results are summarized in Table 1 and illustrated in Figure 1. There was a transient increase in mean arterial blood pressure which subsided within 5–10 minutes after the injury. Intracranial pressure showed small transient elevations which also subsided within a few minutes. Pial arterioles responded to brain injury with vasodilation. The vasodilation was more pronounced in the smaller vessels. It occurred in these smaller vessels at lower levels of injury, at which the larger ones showed no significant change in caliber. Vessel caliber changes reached a steady state 15–30 minutes after the delivery of the blow. In all cats studied, the vasodilation was sustained for the entire period of observation. This arbitrarily was limited to 60 minutes after injury for most experiments. In six cats, all of which were subjected to severe injury in excess of 3 atm, more prolonged systematic observations were made. In these the vasodilation persisted unabated for the entire period of observation (4 hours); at the end of 4 hours, vessel diameter was not significantly different from the corresponding value at 1 hour after injury.
TABLE 1 Mean Arterial Blood Pressure (MABP), Intracranial Pressure (ICP), and Pial Vessel Diameter before and after Fluid Percussion Brain Injury in Five Groups of Cats

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level of injury (atm)</td>
<td>1.4 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>2.5 ± 0.6</td>
<td>2.5 ± 0.1</td>
</tr>
<tr>
<td>Number of cats</td>
<td>9</td>
<td>8</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>Control MABP (mm Hg)</td>
<td>140 ± 8.4</td>
<td>124 ± 4.4</td>
<td>129 ± 7.4</td>
<td>120 ± 6.4</td>
</tr>
<tr>
<td>Control vessel diameter (µm)</td>
<td>176 ± 18.1</td>
<td>52 ± 2.3</td>
<td>167 ± 9.8</td>
<td>56 ± 3.0</td>
</tr>
<tr>
<td>Control ICP (mm Hg)</td>
<td>-1 ± 0.7</td>
<td>-2.5 ± 0.7</td>
<td>-1.1 ± 1.1</td>
<td>0.4 ± 1.3</td>
</tr>
<tr>
<td>Max. post-injury MABP (mm Hg)</td>
<td>166 ± 11.9</td>
<td>151 ± 13.7</td>
<td>216 ± 12.9</td>
<td>218 ± 14.4</td>
</tr>
<tr>
<td>Max. post-injury ICP (mm Hg)</td>
<td>0.5 ± 0.9</td>
<td>2.0 ± 0.7</td>
<td>0.8 ± 1.2</td>
<td>2.8 ± 1.0</td>
</tr>
<tr>
<td>30-min. post-injury MABP (mm Hg)</td>
<td>136 ± 6.4</td>
<td>122 ± 4.5</td>
<td>90 ± 8.8</td>
<td>99 ± 6.5</td>
</tr>
<tr>
<td>30-min. post-injury ICP (mm Hg)</td>
<td>-1.0 ± 0.7</td>
<td>-2.5 ± 0.7</td>
<td>-1.0 ± 1.1</td>
<td>0.4 ± 1.3</td>
</tr>
<tr>
<td>60-min. post-injury MABP (mm Hg)</td>
<td>96 ± 2.6</td>
<td>113 ± 6.3</td>
<td>110 ± 7.3</td>
<td>143 ± 11.5</td>
</tr>
<tr>
<td>60-min. post-injury vessel diameter (% of control)</td>
<td>130 ± 6.6</td>
<td>119 ± 4.2</td>
<td>96 ± 7.1</td>
<td>102 ± 4.4</td>
</tr>
<tr>
<td>60-min. post-injury ICP (mm Hg)</td>
<td>-0.9 ± 0.7</td>
<td>-2.4 ± 0.7</td>
<td>-1.4 ± 0.9</td>
<td>-1.0 ± 1.0</td>
</tr>
<tr>
<td>60-min. post-injury vessel diameter (% of control)</td>
<td>102 ± 3.3</td>
<td>116 ± 5.8</td>
<td>103 ± 4.5</td>
<td>138 ± 10.2</td>
</tr>
</tbody>
</table>

All values are mean ± SE. In groups 1-4, one vessel per cat was studied; in group 5, one small and one large vessel were studied in each cat.

Figures 1 and 2 show the time course of mean arterial blood pressure (MABP), intracranial pressure (ICP), and vessel diameter for small and large arterioles before and for 1 hour after high intensity brain injury. The values shown are mean ± standard error from six cats. The data correspond to group 5 in Table 1. Control vessel diameters before injury are shown in the inset.

**Responses to Arterial Hypocapnia**

The responses to arterial hypocapnia are shown in Figure 2 in relation to the intensity of the injury. Following moderate degrees of injury (2-3 atm) there was a decrease in the vasoconstrictor response to arterial hypocapnia. At higher intensities of injury (>3 atm) there was complete loss of the ability of the vessels to constrict in response to moderate hypocapnia.

**Autoregulatory Adjustments in Vessel Caliber in Response to Arterial Hypotension**

Figures 3 and 4 show the responses of pial arterioles and arteries of different caliber to stepwise decreases in arterial blood pressure induced by bleeding. Caliber changes for vessels of different sizes are shown separately because we found earlier that the responses of normal vessels to hypotension were size-dependent. Following mild injury (1-2 atm), the vessels dilated as the blood pressure decreased, but the responses were reduced in comparison to the responses of vessels of comparable size from cats not subjected to injury studied previously (Kontos et al., 1978). After moderately severe brain injury (2-3 atm), the vessels became completely passive and actually narrowed as the blood pressure decreased.

**Oxygen Consumption of Pial Arterial Wall**

Figure 5 shows the oxygen consumption rates of pial arterioles from cats with mild (1-2 atm) or moderately severe (2-3 atm) brain injury in relation to the oxygen consumption rates of vessels of comparable size from cats not subjected to injury. There was a decrease in the oxygen consumption rate following severe head injury, but oxygen consump-
CEREBRAL MICROCIRCULATION IN BRAIN INJURY/Wei et al.

Trauma 2.71 ± 0.07 atm

Trauma 3.37 ± 0.11 atm

FIGURE 2  Responses of pial arteriolar diameter to hypocapnia for small and large vessels before and after brain injury. Two groups of six cats each are shown; one group was subjected to moderate intensity, and the second group to heavy intensity injury. The lower portion of the figure shows the absolute values of vessel diameter. The upper part of the figure shows the percentile changes in diameter induced by hypocapnia. Statistical analysis was carried out by comparing with a t-test the percentile change in diameter induced by hypocapnia in each group of vessels before and after injury. In all instances the response to hypocapnia was significantly reduced after injury (P < 0.05). Means ± SE for Paco₂ were as follows: Moderate intensity injury group: before injury, 31 ± 1.7 and 18 ± 0.1 mm Hg for normocapnia and hypocapnia, respectively; after injury, 32 ± 2.3 and 18 ± 1.1 mm Hg. Heavy intensity injury group: before injury, 33 ± 4.7 and 19 ± 2.3 mm Hg; after injury, 32 ± 2.9 and 18 ± 1.6 mm Hg.

Platelet Aggregation

Rosenblum and El-Sabban (1977) found that injury to cerebral vessels of the mouse induced by ultraviolet light in the presence of a sensitizing dye induced platelet aggregation. Because of this finding, we made systematic observations for 2-3 hours after head injury in six cats. There were no platelet aggregates (white bodies) in either arteries or veins in five of these. In the sixth, which had developed severe hypotension after injury (mean arterial blood pressure equal to 57 mm Hg), there were platelet aggregates. Because of the observations in this one, we deliberately induced severe hypotension (mean arterial blood pressure equal to 60 mm Hg) at the end of the observation period in two other cats, but no aggregates appeared. Also, there were no platelet aggregates observed in the pial, intracortical, or brainstem vessels examined histologically by electron microscopy.

Electron Microscopic Observations

The gross morphological findings for the brain after injury were the same as those reported previously by Sullivan et al (1976), using the same technique. In the cortex, there were no visible lesions...
after low intensity (<2 atm) injury. After more severe injury, there was hemorrhagic contusion limited to the site of implantation of the metal shaft connecting the cranium to the fluid percussion device.

Scanning electron microscopic examination of the luminal surface of pial arterioles from control cats revealed a smooth endothelial surface (Fig. 6A). The endothelial cells were observed as being fusiform in shape and oriented with their long axis parallel to the long axis of the vessel. Ovoid protrusions representing underlying nuclei were observed frequently, as were marginal folds along the cell borders. Transmission electron microscopic examination of adjacent vessel segments revealed morphological characteristics (Fig. 6B) consistent with those previously described by Pease and Molinari (1960).

In pial arterioles from mechanically injured cats, scanning electron microscopy demonstrated two types of lesions on the vessels’ luminal surfaces. Numerous crater-shaped indentations or defects in the endothelial surface of the pial vasculature could be visualized readily (Fig. 6C). Such craters varied considerably in size (0.2-7 μm) and shape. Most of them were circular and measured 3-5 μm in diameter. In addition to these craters, numerous dome-shaped projections of the endothelial cell surface or “balloons” were also observed (Fig. 6C). As in the case of the craters, these lesions varied considerably in both size and shape. Transmission electron microscopy conducted on the adjacent vascular segments of the same pial arterioles confirmed and supplemented those observations made with the scanning electron microscope. Cytoplasmic vacuoles frequently were visualized within endothelial cells, and occasionally such vacuoles protruded into the lumen forming cytoplasmic blebs (Fig. 6D). Concavities or indentations of the endothelial surface (Fig. 6E) also were observed commonly. At these sites the endothelial membrane very frequently was clearly necrotic. Such concavities and cytoplasmic blebs observed with transmission electron microscopy were assumed to correspond, respectively, to the craters and balloons noted in the scanning electron microscopic analysis. Transmission electron microscopy revealed that the tight junctions between adjacent endothelial cells were intact; however, junctional vacuolation was a frequent occurrence. Increased pinocytosis on both
the luminal and abluminal endothelial surfaces was also a consistent feature of these pial arterioles. Despite these endothelial changes within the pial vessels, transmission electron microscopy failed to demonstrate pronounced alterations within either the underlying vascular smooth muscle cells or adventitial coat. The only alteration in either the media or adventitia was the appearance of inclusions within the smooth muscle cells of the media. Such inclusions were infrequent and appeared as electron-lucent amorphous masses encompassed by two unit membranes. Neither cytoplasmic separation from the plasma membrane nor aggregation of thick filaments into ribbon-like structures was seen. These abnormalities have been described in vascular smooth muscle that has been mechanically disrupted by stretching in vitro (Somlyo et al., 1971).

None of the pial arterioles that were obtained from control cats and had normal responses to hypocapnia had any endothelial lesions. On the other hand, all pial arterioles that were obtained from mechanically injured cats and that showed absent or reduced responses to hypocapnia had endothelial lesions. Occasional vessels from mechanically injured cats, which did not dilate and which maintained normal responsiveness to hypocapnia, had no lesions. We quantified the number of lesions by counting the number of dome-shaped projections and craters in five randomly selected microscopic fields, each approximately 2100 μm². Several factors were found to affect the number of lesions present in these vessels. Vessels larger than 100 μm in diameter had 16 ± 1.7 lesions per 2100 μm², whereas vessels less than 100 μm in diameter had 21 ± 1.8 lesions. Vessels from cats subjected to injury at an intensity in excess of 3 atm had more lesions (18 ± 1.4) than vessels from cats subjected to injury at less than 3 atm (6.5 ± 1.5). The responsiveness of the vessels after injury was also considered in relation to the number of lesions. We compared the number of lesions in vessels that were dilated and totally unresponsive to hypocapnia to the number of lesions in vessels that were dilated but somewhat responsive to hypocapnia. In any one cat, the latter group of vessels had fewer lesions than the former. However, this relationship did not hold when comparisons were made between different cats, and considerable overlap existed under these circumstances. Some vessels following head injury dilated in an irregular fashion so that they assumed a sausage-shaped appearance with markedly dilated portions alternating with less dilated

![Figure 6](image-url)
sequences. In these vessels there was a periodicity of the number of lesions, so that the markedly dilated areas had more lesions than the less dilated ones.

In rare instances, we found extensive necrosis and destruction of endothelial cells (Fig. 7). Such extensive lesions were found in smaller (<100 μm in diameter), markedly dilated arterioles obtained from cats subjected to severe (>3 atm) head injury.

Intracortical arterioles and arterioles in the brainstem from cats following brain injury had endothelial lesions similar to those found in the pial vessels.

Effect of Eliminating the Transient Rise in Blood Pressure Immediately After Head Injury

Since similar histological, metabolic, and physiological alterations occur in the pial vessels following acute elevations in arterial blood pressure induced by the intravenous administration of vasoconstrictor agents, such as angiotensin and norepinephrine, as those observed here (Kontos et al., 1978; Navari et al., 1977), we thought that the transient rise in blood pressure seen immediately after head injury might be responsible for the pial arteriolar changes. To investigate this possibility, we prevented the rise in blood pressure following severe head injury in six cats. This was accomplished by connecting the descending aorta with a wide-bore tubing to a reservoir filled with heparinized saline and set at a height corresponding to the resting arterial blood pressure. In addition to this, in some cats it was necessary to administer intravenously a vasodilator agent to prevent completely the rise in blood pressure. For this purpose we used adenosine triphosphate (ATP), because we found in earlier experiments that this agent given intravenously does not have a direct effect on the pial vessels (Kontos et al., 1978), probably because it does not penetrate the blood-brain barrier in sufficient concentration. As shown in Figure 8, the pial arterioles from cats subjected to brain injury, in which the rise in blood pressure was eliminated, did not dilate and remained responsive to arterial hypocapnia. Electron microscopy of these vessels in five cats showed normal histological appearance without any endothelial lesions.

Discussion

The results reported above show that experimental fluid-percussion brain injury in the cat causes functional, morphological, and metabolic abnormalities in the pial arterioles and arteries. The

Figure 7  Transmission electron micrograph of a pial arteriole from a cat 10 minutes after 2.3 atm mechanical brain injury showing extensive endothelial destruction. 7,500X.
transient rise in arterial blood pressure immediately following mechanical injury is essential for the production of these abnormalities. This is clearly demonstrated by the fact that, if this rise in blood pressure is eliminated, the vessels do not dilate, remain responsive, and appear normal morphologically. Additional evidence incriminating the rise in blood pressure in the production of these abnormalities is the fact that if the arterial blood pressure is raised by the intravenous administration of vasoconstrictor agents, such as angiotensin or norepinephrine, identical functional, metabolic, and morphological abnormalities are found in the pial arterioles and arterioles (Kontos et al., 1978; Navari et al., 1977). The rise in blood pressure induced by mechanical brain injury most likely is due to increased sympathetic activity and consequent peripheral vasostriction related to pressure or hemorrhage in the brainstem as a result of the increased intracranial pressure associated with the pressure pulse of the fluid percussion (Denny-Brown and Russell, 1941).

Histological lesions in the endothelium similar to those we reported here have been demonstrated by others in cerebral vessels in SHR rats with sustained hypertension (Hazama et al., 1978) and in cerebral vessels in response to ischemia (Nelson et al., 1975). In addition, similar lesions associated with a reduction in resting oxygen consumption rates have been found in the rabbit aorta in vitro following hypoxia (Morrison et al., 1977). Our observations suggest that these lesions begin as discrete areas of destruction in the endothelium that manifest themselves as vacuolization, which gives rise to the dome-shaped lesions or balloons. If these progress to rupture of the surface membrane of the endothelium, they become craters. When the density of these lesions is large enough, then more extensive areas of the endothelium are destroyed and disrupted. In such cases there is evidence of extensive endothelial necrosis.

The mechanism by which the rise in blood pressure produces these lesions is not known. Fry (1969) suggested mechanisms by which the rise in blood pressure could induce entry of various molecules from the blood stream into the endothelium. His considerations pertained primarily to macromolecules, such as plasma proteins. The same considerations could apply equally well to smaller molecules, such as calcium ions. If a large increase in the flux of calcium ions into the endothelial cells occurred, one would expect the activation of a larger number of enzymatic reactions which could conceivably produce agents injurious to the cell and result in localized destruction. We have preliminary evidence that implicates increased production of prostaglandins in the production of these lesions. Animals pre-treated with cyclooxygenase inhibitors did not display any of the morphological or functional abnormalities we found following mechanical brain injury in untreated animals, despite the fact that the rise in arterial blood pressure after brain injury was unaffected. In this connection, the absence of platelet aggregation in the face of extensive endothelial damage is noteworthy. It suggests strongly the possibility that substances which inhibit platelet aggregation, such as prostacyclin, may be produced following experimental brain injury. Hornstra et al. (1978) found that endothelial damage increased the release of prostacyclin-like material from rat and rabbit aortas.

The close association of the endothelial lesions with the functional abnormalities of the vascular smooth muscle is of interest. This is demonstrated by the fact that dilated and unresponsive vessels always had endothelial lesions, whereas vessels that remained normally responsive and did not dilate did not have them. Similarly, the largest number of lesions was found in the most severely damaged vessels, and the unevenly dilated vessels had the greatest density of lesions in the most dilated por-
tions. This close association suggests a cause and effect relationship. One reasonably could surmise that the endothelial lesions initially are produced by the increased blood pressure and then release vasodilator substances which could induce vascular smooth muscle relaxation. The pial arteriolar vasodilation, the inability of the pial vessels to undergo further autoregulatory dilation in response to a decrease in arterial blood pressure, and the reduced or absent responsiveness to the vasoconstrictor effect of arterial hypocapnia clearly are consequences of vascular smooth muscle relaxation. Another potential mechanism for the induction of relaxation of the pial vascular smooth muscle after injury is mechanical stretch of the vessels during the transient hypertensive episode following brain injury. Dobrin (1973) found that activation of vascular segments of dog carotid artery at high distending pressures resulted in decrease in strength or number of contractile units in the vessel wall. This diminished ability of the vascular smooth muscle to generate tension under these conditions might have been due to rupture of attachments of the myofilaments to the plasma membrane (Somlyo et al., 1971). We found no morphological evidence of mechanical disruption of pial arterioles or arteries after brain injury. We, therefore, do not believe that mechanical disruption was an important factor in bringing about arteriolar muscle relaxation after brain injury.

We consider the reduced oxygen consumption of the vessel wall as the metabolic counterpart of the damage to the vascular wall. It is reasonable to expect that, at least in part, this reduction in oxygen consumption rates is caused by reduced oxygen consumption of the endothelium, since endothelial cells display indisputable evidence of morphological damage. However, the endothelium is only a small portion of the total mass in the vascular wall, and it is likely that the substantially reduced oxygen consumption rate also reflects a reduction in the oxygen consumption of the other components of the vessel wall, namely the vascular smooth muscle. It is of interest that others (Morrison et al., 1977) found that hypoxia reduces the oxygen consumption of rabbit aortic tissue in vitro and, at the same time, produces endothelial lesions similar to the ones we reported here. It is possible that the decreased oxygen consumption rate may represent a metabolic effect of substances released by the damaged endothelium, or it may reflect injury to the vascular smooth muscle that may not be apparent on histological examination.

Our main aim in this work has been to provide morphological, functional, and metabolic correlations in the same vessels. For this reason, detailed observations were limited to the pial vessels which are the only ones that can be studied visually. We do know, however, from limited morphological observations of intracortical and brain stem vessels, that the same endothelial lesions occur in these vessels as in the pial arteries and arterioles. It will be of interest to identify functional abnormalities in these vessels similar to those we described for the pial vessels. Necessarily, this will have to be done by indirect methods using pressure and flow measurements.

Of interest is the fact that the lesions in the brainstem described elsewhere (Povlishock et al., 1978), consisting of increased pinocytotic transport of protein across the vessel wall, are not affected by the control of the blood pressure and must, therefore, have a different mechanism (Povlishock et al., 1979). We evidently are dealing with at least two different types of vascular lesions, one due to the rise in arterial blood pressure and the other independent of it.

There are many studies of the alterations in cerebral blood flow in humans following head injury, as well as in animals with various forms of experimental brain injury. The results of these studies are highly variable. Both vasodilation, vasoconstriction and biphasic responses, consisting initially of vasodilation followed by later vasoconstriction, have been reported. The variability in the responses is not known with certainty, but it is understandable if one considers the major differences in the conditions under which these measurements were made. Factors that seem to be important are the timing of the observations with respect to the occurrence of the head or brain injury and the intensity of the injury, as well as the nature of the lesions it induces.

The method we used for the induction of brain injury has certain distinct advantages. The intensity of the injury can be quantified. The pathological lesions, as well as the physiological abnormalities in the pial vessels, bear a reasonably close quantitative relation to the intensity of the injury. The method allows close correlation between morphological lesions and functional abnormalities. These characteristics obviously are desirable for any model of brain injury, but it may be premature at this stage to reach firm conclusions about the possible relevance of this model to human brain injury. This must rely on future studies which show additional similarities to its human counterpart and disclose its pathogenesis more fully. If such relevance is demonstrated, it is not likely that this would be a model for all forms of human head injury, because of the diversity of the lesions in the latter. It is more likely to mimic concussive head injury in which no gross lesions are seen or head injury which involves hemorrhage in the brain stem and subarachnoid hemorrhage.

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