Role of endothelin-1 in age-dependent cerebrovascular hypotensive responses after brain injury

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Armstead, William M. Role of endothelin-1 in age-dependent cerebrovascular hypotensive responses after brain injury. Am. J. Physiol. 274 (Heart Circ. Physiol. 46): H1884–H1894, 1999.—This study was designed to compare the effect of fluid percussion brain injury (FPI) on the hypertensive cerebrovascular response in newborn and juvenile pigs as a function of time postinsult and to determine the role of endothelin-1 (ET-1) in any age-dependent differences in hypertensive cerebrovascular regulation after injury. Ten minutes of hypotension (10–15 ml blood/kg) decreased mean arterial blood pressure uniformly in both groups (~45%). In the newborn, hypertensive pial artery dilation (PAD) was blunted within 1 h, remained diminished for at least 72 h, but was resolved within 168 h postinjury (66 ± 4, 69 ± 4, 71 ± 4, and 64 ± 4% inhibition at 1, 4, 8, and 72 h post-FPI). During normotension, regional cerebral blood flow (rCBF) was decreased by FPI, and hypertension further reduced the already decremented rCBF for at least 72 h. Cerebrospinal fluid (CSF) ET-1 was increased from 26 ± 4 to 206 ± 25 pg/ml within 72 h post-FPI, whereas an ET-1 antagonist partially restored impaired hypertensive PAD and altered hypotensive rCBF. In contrast, hypertensive PAD and altered CBF were only inhibited for 4 h post-FPI in the juvenile (56 ± 3 and 34 ± 4% inhibition at 1 and 4 h post-FPI). CSF ET-1 was only increased from 27 ± 4 to 67 ± 9 pg/ml at 4 h, whereas the concentration returned to preinjury value by 8 h post-FPI. ET-1 antagonism similarly partially restored impaired hypertensive PAD and altered hypotensive rCBF. These data show that FPI disturbs cerebral autoregulation during hypotension both to a greater magnitude and for a longer duration in the newborn than in the juvenile. These data suggest that the greater FPI-induced ET-1 release in the newborn could contribute to age-dependent differences in impaired hypertensive cerebral autoregulation after FPI.

newborn; cerebral circulation; hemorrhagic hypotension

TRAUMATIC BRAIN INJURY is one of the major causes of morbidity, mortality, and pediatric intensive care unit admissions of children today (35, 38). Although the effects of traumatic brain injury have been well described for adult animal models (15, 27, 28, 42), few have investigated these effects in the newborn or have characterized such effects as a function of age using a single model of injury. For example, Adelson et al. (1) described the motor and cognitive functional deficits following diffuse traumatic brain injury using a weight drop model in the immature rat. Similarly, Smith et al. (37) described the role of oxygen free radicals in brain injury using a newly characterized infant rat model of the shaken baby syndrome. To reproduce some of the biomechanical aspects of closed head injury, fluid percussion brain injury (FPI) has been used in the adults of several species (27, 28). More recently, Prins et al. (34) characterized the effects of FPI on several parameters including mortality, intracranial pressure, and mean arterial blood pressure in the developing and adult rat. Other earlier studies had compared the cerebral hemodynamic effects of FPI in newborn (1–5 days old) and juvenile (3–4 wk old) pigs. For example, it was observed that pial vessels constricted more, and that regional cerebral blood flow decreased and remained depressed longer, in newborns than in juveniles (9). Moreover, systemic arterial blood pressure increases in the juvenile pig after brain injury, consistent with other adult studies (42), whereas it decreases in the newborn pig (9). Whereas the latter studies did characterize several hemodynamic parameters as a function of age by using the same injury model, these studies were restrictive in the time period investigated postinsult (3 h). Equally important, the above studies did not investigate the physiological significance and mechanisms for such age-related differences post-FPI.

Hypotension, which is present in 15–35% of severe head injury patients, increases mortality and morbidity (11, 26). After traumatic brain injury, ischemic brain injury may result from reduced cerebral perfusion pressure due to intracranial hypertension or to the inability of the cerebral circulation to respond to hypotension after the insult. In experimental models of traumatic brain injury, the normal cerebral autoregulatory response was impaired in the adult cat (16, 25). However, few studies have examined the mechanism for such impairment or characterized the effects of brain injury on the cerebrovascular response to hypotension in the newborn.

In the newborn pig, hypertensive pial artery dilation has been observed to be associated with increased cortical periarachnoid cerebrospinal fluid (CSF) prostaglandin concentration, whereas the cyclooxygenase inhibitor indomethacin blunted the pial dilation, indicating a role for prostaglandins in such dilation (23, 24). More recent data suggest that, in fact, such dilation during hypotension results from the sequential release of prostaglandins and cAMP, which, in turn, activates both the ATP-sensitive K⁺ (KATP) and calcium-sensitive K⁺ (KCa) channels (3), ionic mechanisms thought to be important in the regulation of cerebrovascular tone (22, 31, 32). In the newborn pig, FPI has been observed to impair KATP and KCa channel function (4, 5). In separate experiments under non-brain injury conditions, it was also observed that coadministration of the peptide endothelin-1 (ET-1) with KATP-channel agonists blunted...
dilation to such stimuli, suggesting that this peptide interacts with and impairs the K<sub>ATP</sub> channel (21). Because the CSF concentration of ET-1 was increased after FPI (8), and an antagonist for ET-1, BQ-123, partially restored decremented dilation to K<sub>ATP</sub>-channel agonists after FPI in the newborn pig (21), ET-1 appears to contribute to altered cerebral hemodynamics after FPI through impairment of K<sub>ATP</sub>-channel function.

Therefore, the present study was designed to compare the effect of FPI on the cerebrovascular response to hypotension in newborn and juvenile pigs as a function of time postinjury and to determine the role of ET-1 in altered hypotensive cerebrovascular regulation.

METHODS

Newborn (1–5 days old, 1.3–2.1 kg) and juvenile (3–4 wk old, 6.0–8.3 kg) pigs of either sex were used in these experiments. All protocols were approved by the Institutional Animal Care and Use Committee. For acute experiments, animals were anesthetized with ketamine hydrochloride (33 mg/kg) and acepromazine (3.3 mg) intramuscularly. Anesthesia was maintained with α-chloralose (30–50 mg/kg, supplemented with 5 mg/kg per hour i.v.). A catheter was inserted into a femoral artery to monitor blood pressure and to sample for blood gas tensions and pH. Drugs to maintain anesthesia were administered through a second catheter placed in a femoral vein. The trachea was cannulated, and the animals were mechanically ventilated with room air. A heating pad was used to maintain the animals at 37–39°C.

A cranial window was placed in the parietal skull of these anesthetized animals. This window consisted of three parts: a stainless steel ring, a circular glass coverslip, and three posts consisting of 17-gauge hypodermic needles attached to three precut holes in the stainless steel ring. For placement, the dura was cut and retracted over the cut bone edge. The cranial window was placed in the opening and cemented in place with dental acrylic. The volume under the window was filled with a solution, similar to CSF, of the following composition (in mM): 3.0 KCl, 1.5 MgCl<sub>2</sub>, 1.5 CaCl<sub>2</sub>, 132 NaCl, 6.6 urea, 3.7 dextrose, and 24.6 NaHCO<sub>3</sub>. This artificial CSF was warmed to 37°C and had the following chemistry: pH 7.33, PCO<sub>2</sub> 46 mmHg, and PO<sub>2</sub> 43 mmHg, which was similar to that of endogenous CSF. Pial arterial vessels were observed with a dissecting microscope, a television camera mounted on the microscope, and a video output screen. Vascular diameter was measured with a video microscaler.

Methods for brain FPI have been described previously (42). A device designed by the Medical College of Virginia was used. A small opening was made in the parietal skull contralateral to the cranial window. A metal shaft was sealed into the opening on top of intact dura. This shaft was connected to the transducer housing, which was in turn connected to the fluid percussion device. The device itself consisted of an acrylic plastic cylindrical reservoir 60 cm long, 4.5 cm in diameter, and 0.5 cm thick. One end of the device was connected to the transducer housing, whereas the other end had an acrylic plastic piston mounted on O-rings. The exposed end of the piston was covered with a rubber pad. The entire system was filled with 0.9% saline. The percussion device was supported by two brackets mounted on a platform. FPI was induced by striking the piston with a 4.8-kg pendulum. The intensity of the blow (usually 1.9–2.3 atm with a constant duration of 19–23 ms) was controlled by varying the height from which the pendulum was allowed to fall. The pressure pulse of the blow was recorded on a storage oscilloscope triggered photoelectrically by the fall of the pendulum. The amplitude of the pressure pulse was used to determine the intensity of the injury.

For chronic survival surgery experiments designed to investigate the effects of FPI on pigs 72 and 168 h postinsult, pigs were anesthetized with isoflurane and an aseptic technique was used to surgically place the adapter for connection to the brain injury device in the skull of the animal. After injury was induced, the animal was allowed to recover, and the techniques for cranial window placement described above and the experimental protocol described below were performed 72 or 168 h post-FPI.

Regional blood flow distribution within the brain was measured using radioactively labeled microspheres. We have used this method to measure blood flow in both anesthetized and conscious animals under a variety of experimental conditions. Briefly, a known amount of radioactivity in 15-µm microspheres (300,000–800,000 spheres) was injected into the left ventricle, and the injection line was flushed with 1 ml of saline. Withdrawal of reference blood samples was begun 15 s before microsphere injection and continued for 2 min after the injection. The reference withdrawal rate was 1.03 ml/min. After each experiment, the pig was killed and the brain removed and weighed. The brain was subdivided into major regions, and samples were counted in a gamma counter. The energy from each nuclide was separated by differential spectroscopy. Aliquots of the actual microsphere solutions injected were used for overlap calculations. The count in each milliliter per minute of blood flow was determined by dividing the counts in the reference withdrawal by the rate of reference withdrawal. Thus blood flow to any organ at the time the microspheres were injected can be calculated as

\[ Q = C \times R \times CR \]

where \( Q \) is organ blood flow (in ml/min), \( C \) is counts per minute (cpm) in the tissue sample, \( R \) is the rate of withdrawal of reference blood sample (in ml/min), and \( CR \) is the total counts in the reference arterial blood sample. Regional blood flow data reflect flow to structures both ipsilateral and contralateral to the injury site.

Protocol. Two types of pial arterial vessels, small arteries (nesting diameter 120–160 µm) and arterioles (nesting diameter 50–70 µm), were examined to determine whether segmental differences in the effects of FPI on hypotensive pial dilation could be identified. Pial arterial vessel diameter was determined every minute for a 10-min exposure period after infusion onto the exposed parietal cortex of artificial CSF before hypotension and after the infusion of artificial CSF after the induction of hypotension. Typically, 2–3 ml of CSF were flushed through the window over a 30-s period, and excess CSF was allowed to run off through one of the needle ports. For sample collection, 300 µl of the total cranial window volume of 500 µl was collected by slowly infusing CSF into one side of the window and allowing the CSF to drip freely into a collection tube on the opposite side.

Twelve types of experiments were performed: 1) newborn acute FPI (<8 h postinsult; n = 6), 2) newborn chronic FPI (72 h postinsult; n = 6), 3) newborn chronic FPI (168 h postinsult; n = 6), 4) newborn BQ-123-pretreated acute FPI (<8 h postinsult; n = 6), 5) newborn BQ-123-pretreated chronic FPI (72 h postinsult; n = 6), 6) newborn BQ-123-pretreated chronic FPI (168 h postinsult; n = 6), 7) juvenile acute FPI (<8 h postinsult; n = 6), 8) juvenile BQ-123-pretreated acute FPI (<8 h postinsult; n = 6), 9) time control newborn acute FPI (8 h; n = 6), 10) time control newborn chronic FPI (72 h; n = 6), 11) time control newborn chronic FPI (168 h; n = 6), and 12) time control juvenile acute FPI (8 h; n = 6). Small
arteries and arterioles were observed in each of these experimental groups.

Hypotension was induced by rapidly withdrawing 10–15 ml blood/kg, which decreased mean arterial blood pressure to 35–40 mmHg. The drop in blood pressure was designed to be an ~45% decrease in pressure from the resting control value in all experimental groups. Such a drop in mean blood pressure was maintained constant for 10 min by titration of additional blood withdrawal (1–5 ml) to keep arterial blood pressure from rising and by blood reinfusion (1–5 ml) to keep pressure from falling. The percent changes in artery diameter values were calculated on the basis of the diameter measured in the control period before hypotension.

Responses to hypotension and the constrictor peptide ET-1 (10^{-8} and 10^{-6} M, Sigma) were obtained before and after FPI in the absence and presence of BQ-123 (RBI). Time control experiments were designed so that responses were obtained initially and then again at 1, 4, and 8 h in acute sham control experiments, whereas such responses were only obtained at 72 and 168 h after sham control surgery in chronic surgery animals. In BQ-123-pretreated animals, this antagonist was administered at a concentration of 1 mg/kg intravenously in acute and 1 mg/kg intraperitoneally in chronic surgery experiments. In both acute and chronic surgery experiments, 10^{-6} M topical BQ-123 was also administered during the hypotensive experimental protocol post-FPI to further make certain that ET-1-receptor blockade was adequate. Confirmation of ET-1-receptor blockade was determined by comparing responses to ET-1 before and after BQ-123 administration (1, 4, 8, 72, and 168 h postinsult or sham control surgery).

Endothelin-1 analysis. The CSF samples that were collected were rapidly frozen and stored at ~20°C. Radioimmunoassay kits for ET-1 are commercially available (Peninsula, Belmont, CA). Radioimmunoassay uses the simultaneous addition of sample, rabbit anti-endothelin antibody, and the 125I-labeled derivative of ET-1. After an overnight incubation at 4°C, free ET-1 was separated from ET-1 bound to antibody by the addition of goat anti-rabbit immunoglobulin G serum and normal rabbit serum. After centrifugation at 760 g for 10 min, the supernatant was decanted and the pellet counted using a gamma scintillation counter. All sample and standards were assayed in duplicate. Data are calculated as \%B/Bo versus concentration, where \%B/Bo = (average cpm of sample − average cpm of nonspecific binding tube)/Bo × 100 and Bo = average cpm of total binding tube − average cpm of nonspecific binding tube.

Statistical analysis. Pial arteriolar diameter, systemic arterial pressure, and ET-1 levels were analyzed using analysis of variance for repeated measures or t-test where appropriate. Data were normally distributed. If the value was significant, the data were then analyzed by Fisher’s protected least significant difference test. An alpha level of P < 0.05 was considered significant in all statistical tests. Values are represented as means ± SE of the absolute values or percentages of change from control values.

**RESULTS**

Influence of FPI on pial artery dilation during hypotension as a function of time in the newborn pig. Experiments were initially designed to show that pial artery responses to hypotension were reproducible over time in two different sham control preparations. In the first preparation, agonist responses were obtained initially (0 h) and at 1, 4, and 8 h in an acute sham control preparation (cranial window and brain injury adapter placed in the skull but no injury induced). In the second preparation, chronic or survival surgery (injury adapter placed in the skull and then withdrawn without injury being induced, followed by recovery and placement of cranial window 72 or 168 h later) pial responses during hypotension were obtained 72 or 168 h after sham surgery and compared with responses obtained initially (0 h) in the acute preparation. Summary data for both sham control preparations are shown in Table 1. These data show that responses obtained at 1, 4, 8, 72, and 168 h were no different from those obtained initially (0 h) (Table 1). On a percentage basis, mean arterial blood pressure was decreased to an equivalent extent at each time point (47 ± 3, 45 ± 3, 48 ± 3, 46 ± 3, 47 ± 2, and 48 ± 3% for 0, 1, 4, 8, 72, and 168 h, respectively). In contrast, pial artery dilation during hypotension was blunted within 1 h after FPI (Fig. 1). Such diminished vascular responsiveness was maintained for at least 72 h but was resolved within 168 h postinjury (Fig. 1). On a percentage basis, hypotensive pial small artery and arteriole dilation were inhibited by 66 ± 4 and 66 ± 5% at 1 h post-FPI, 69 ± 4 and 72 ± 5% at 4 h post-FPI, 71 ± 4 and 68 ± 6% at 8 h post-FPI and 64 ± 4 and 58 ± 6% at 72 h post-FPI, respectively.

Role of ET-1 in altered pial artery dilation during hypotension after FPI in the newborn pig. Cortical periarachnoid CSF ET-1 concentration was increased at 1, 4, 8, and 72 h post-FPI but returned toward control (preinjury) value within 168 h post-FPI (26 ± 4, 98 ± 10, 234 ± 37, 286 ± 47, 206 ± 25, and 35 ± 5 pg/ml ET-1 during preinjury and at 1, 4, 8, 72, and 168 h post-FPI, respectively; n = 6). In animals pretreated with the ET-1 antagonist BOQ-123, pial artery dilation during hypotension was not decremented as greatly as in the absence of this antagonist (Fig. 1). The ability of BQ-123 to partially protect such responsiveness lasted through 72 h post-FPI (Fig. 1). Confirmation of the effectiveness of ET-1-receptor blockade was performed by obtaining responses to topical ET-1 at 1, 4, 8, and 72 h post-BQ-123 administration in FPI animals and comparing these vascular responses to those in the absence of BQ-123. In all cases, blockade was >85% (e.g., 10^{-8} M ET-1 produced 18 ± 2% constriction in vehicle animals, whereas 10^{-8} M ET-1 produced 3 ± 1% constriction at 1 h after BQ-123 administration). Additionally, BQ-123 blocked responses to ET-1 in the absence of FPI. BQ-123 itself had no effect on pial artery diameter.

Effect of FPI on autoregulation of cerebral blood flow during hypotension as a function of time in the newborn pig: role of ET-1. During normotension,
FPI produced reduced regional cerebral blood flow, and such reductions lasted for at least 72 h (Figs. 2–5). Although not noted on Figs. 2–5, such reductions were bilaterally equivalent; therefore, no differences between flow on the side ipsilateral and that on the side contralateral to the impact site were noted at any time points (e.g., cerebral cortical blood flow fell from $52 \pm 5$ to $23 \pm 3 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ g}^{-1}$ ipsilateral and from $50 \pm 5$ to $28 \pm 4 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ g}^{-1}$ contralateral to the impact site within 1 h after FPI). By 168 h, however, cerebral
blood flow had returned toward control value (Figs. 2–5).

Whereas hypotension did not alter cerebral blood flow before injury (0 h), indicating intact cerebral autoregulation, regional cerebral blood flow fell to lower levels during hypotension than during normotension at 1, 4, 8, and 72 h post-FPI (Figs. 2–5). Although not shown in Figs. 2–5, the decrease in blood flow during hypotension compared with that during normotension was equivalent bilaterally. However, FPI did not decrease cerebral blood flow compared with that during normotension at 168 h post-FPI (Figs. 2–5).

Reductions in regional cerebral blood flow elicited by FPI as a function of time during normotension and hypotension were fairly uniform in the eight brain regions investigated (Figs. 2–5). Mean arterial blood pressure was decreased to an equivalent extent at all time points.

In another series of experiments, the role of ET-1 in disturbed cerebral autoregulation after FPI was investigated. BQ-123 pretreatment during normotension had no effect on cerebral blood flow before injury (0 h) but attenuated brain injury-induced reductions in cerebral blood flow at 1, 4, 8, and 72 h post-FPI (Figs. 2–5). BQ-123 pretreatment similarly did not affect cerebral blood flow during hypotension before injury (0 h) (Figs. 2–5). However, BQ-123 pretreatment did attenuate reductions in cerebral blood flow during hypotension compared with during normotension at 1, 4, 8, and 72 h post-FPI (Figs. 2–5).

Influence of FPI on pial artery dilation during hypotension as a function of time. Experiments were designed to investigate the effects of brain injury on the ability of hypotension to elicit pial artery dilation as a function of time postinjury in the juvenile pig. Results of these experiments show that pial dilation induced by hypotension was attenuated at 1 and 4 h post-FPI but that responsiveness returned toward control value within 8 h post-FPI (Fig. 6). On a percentage basis, hypotensive pial small artery and arteriole dilation were inhibited by 56 ± 3% and 54 ± 4% at 1 h post-FPI and 34 ± 4 and 47 ± 5% at 4 h post-FPI, respectively.

CSF ET-1 concentration was increased at 1 and 4 h post-FPI but returned toward control value (preinjury) value within 8 h post-FPI (27 ± 4, 67 ± 9, 47 ± 6, and 35 ± 5 pg/ml ET-1 during preinjury and at 1, 4, and 8 h post-FPI, respectively, n = 6). BQ-123 pretreatment partially restored hypotensive pial dilation similar to that in the newborn pig (Fig. 6). Topical ET-1-induced vasoconstriction was blunted for ≤8 h after topical administration of BQ-123, similar to that in the newborn pig. BQ-123 by itself had no effect on pial artery diameter.
Effect of FPI on autoregulation of cerebral blood flow during hypotension as a function of time postinjury in the juvenile pig. During normotension, FPI reduced regional cerebral blood flow 60 min postinjury, but such reductions returned toward control (preinjury) value for all regions except the cerebrum within 4 h postinjury (Figs. 7 and 8). Altered blood flow within the cerebrum returned to preinjury value within 8 h postinjury (Fig. 7). Whereas hypotension did not alter regional cerebral blood flow before injury (0 h), such flow fell to lower levels during hypotension than during normotension at 1 h post-FPI in all regions (Figs. 7 and 8). Within 4 h, however, hypotension no longer altered blood flow in any regions expect the cerebrum. Within 8 h post-FPI, the blood flow in the cerebrum was also no different during hypotension than during normotension (Fig. 7).

Blood chemistry, mean arterial blood pressure, and intensity of injury. Blood chemistry values were obtained at the beginning and end of all experiments. These values were 7.45 ± 0.01, 36 ± 3 mmHg, and 93 ± 5 mmHg versus 7.43 ± 0.01, 35 ± 3 mmHg, and 90 ± 5 mmHg for pH, Pco2, and P02, respectively, before and after injury in acute (8 h) experiments. Similar values were obtained for chronic survival surgery pigs at 72 and 168 h postinjury. Hypotension decreased mean arterial blood pressure from 71 ± 5 to 39 ± 4 mmHg. On a percentage basis, there were no differences in the hypotensive drop in mean arterial blood pressure among time points postinjury as well as among groups (newborn, juvenile, acute, chronic, untreated, BQ-123 pretreated). Finally, the amplitude of the pressure pulse as an index of injury intensity was equivalent in newborn and juvenile animals (2.0 ± 0.1 vs. 2.1 ± 0.1 atm).

**DISCUSSION**

Results of the present study show that pial artery dilation during hypotension was blunted for at least 72 h but returned toward the response observed before injury within 168 h after brain injury in the newborn pig. In contrast, responses to this same stimulus were decremented for only the 1- and 4-h time points post-FPI in the juvenile pig. Furthermore, the magnitude of inhibition at the 1- and 4-h time points post-FPI was significantly greater in the newborn than in the juvenile pig. With the use of vasodilation as an index of activity, these data suggest that cerebral autoregulation was impaired to a greater extent and for a longer time period in the newborn than in the juvenile pig. On the basis of interspecies extrapolation of brain growth curves (17), the age period of newborn pigs chosen in

![Graphs showing the influence of FPI on CBF as a function of time post-FPI during normotension and hypotension in untreated and BQ-123-pretreated animals (n = 6). A: white untreated; B: white BQ-123 pretreated; C: medulla untreated; D: medulla BQ-123 pretreated. *P < 0.05 compared with corresponding preinjury value (0 h). #P < 0.05 compared with corresponding normotension value. &&P < 0.05 compared with corresponding non-BQ-123 pretreated value.](http://ajpheart.physiology.org/10.1152/ajpheart.00746.2016)
the present study may approximate the newborn-to-infant time period in the human. Correspondingly, the age period for the juvenile pig chosen in the present study may correlate to that of a human child 5–8 yr of age (17). Because pial responses to hypotension were reproducible over time in both the newborn and juvenile sham control (acute and chronic) preparations, these data indicate that any alteration of hypotensive pial responsiveness after FPI must be due to the planned insult and not to inherent hemodynamic instability in either an acute or chronic preparation. Concomitantly, the decrease in mean arterial blood pressure

Fig. 5. Influence of FPI on CBF as a function of time post-FPI during normotension and hypotension in untreated and BQ-123-pretreated animals (n = 6). A: pons untreated; B: pons BQ-123 pretreated; C: cerebellum untreated; D: cerebellum BQ-123 pretreated. *P < 0.05 compared with corresponding preinjury value (0 h). †P < 0.05 compared with corresponding normotension value. ‡P < 0.05 compared with corresponding non-BQ-123 pretreated value.

Fig. 6. Influence of FPI on hypotension-mediated pial small artery (A) and arteriole dilation (B) as a function of time post-FPI in the juvenile pig in absence (control) and presence of BQ-123 (n = 6). *P < 0.05 compared with preinjury value (0 h). †P < 0.05 compared with control.
during hypotension, when compared on a percentage basis, was equivalent in all groups at all time points. Finally, the amplitude of the pressure pulse, which reflects the intensity of the injury, was equivalent in newborn and juvenile pig groups. However, how this force acts once it enters the skull may depend on the differences in the composition and compliance of the newborn and juvenile brain. Additionally, it is unclear how developmental parameters such as brain water content, skull dimensions, or suture elasticity will affect the biomechanics of the fluid wave pulse delivered to the brains of these two age groups (34). Therefore, it remains to be determined whether the lateral FPI technique produces more mechanical damage in the newborn than in the juvenile pig.

Additional results of the present study show that cortical periarachnoid CSF ET-1 concentration was elevated after FPI in both newborn and juvenile pigs. However, the magnitude of the increase and the duration of time during which CSF ET-1 concentration was elevated was greater in the newborn than in the juvenile pig. Pretreatment with the ET-1 antagonist BQ-123 partially restored decremented pial artery dilation during hypotension after FPI in newborn and juvenile pigs. Confirmation of the effectiveness of ET-1 receptor blockade was performed by obtaining responses to ET-1 at 1, 4, 8, and 72 h after BQ-123 administration in FPI animals and comparing these responses to those obtained in the absence of BQ-123. In all cases, blockade was >85%. Because systemically administered BQ-123 blunted responses to topical ET-1 for 72 h after its administration in the newborn pig, these data indicate that BQ-123 crosses the blood-brain barrier in sufficient quantity to inhibit responses to centrally administered ET-1. These observations are in contrast to those of Clozel and Watanabe (12), who found that systemic BQ-123 could not, whereas intracisternal BQ-123 could, prevent the decrease in cerebral blood flow associated with subarachnoid hemorrhage in the adult rat. Whereas reasons for differences between the present study and that of Clozel and Watanabe are uncertain, at least one explanation relates to the idea that the blood-brain barrier is selectively permeable in the newborn period (10). Although FPI may also affect blood-brain barrier permeability, such injury is less likely a reason for the ability of systemic BQ-123 to inhibit responses to topical ET-1 because such observations were similarly made in sham control, non-brain-injured piglets. Because restoration of pial vascular responsiveness was concurrent with elevated CSF ET-1 concentration, these data suggest that ET-1 contributes
to age-dependent differences in impaired pial artery dilation during hypotension after brain injury.

Concomitant with information related to the relative effects of FPI on pial responses to hypotension were the additional results of this study concerning FPI effects on cerebral blood flow autoregulation during hypotension. These results show that during normotension FPI produced reduced regional cerebral blood flow and that such reductions lasted for at least 72 h, but were resolved within 168 h in the newborn pig. In contrast, during normotension cerebral blood flow was decremented in all regions only at 1 h post-FPI in the juvenile pig. At 4 h post-FPI, all regions had returned to preinjury values except for the cerebrum, which, in turn, returned to its preinjury value within 8 h. These data extend those of a previous study (9) that only compared the effects of FPI in newborn and juvenile pigs for the first 3 h postinsult. Results of both studies, however, are consistent with the idea that the newborn is more sensitive to brain injury. New data from this study show that elevated CSF ET-1 contributes to reductions in cerebral blood flow after FPI because the ET-1 antagonist BQ-123 attenuated brain injury-induced reductions in cerebral blood flow. Whereas hypotension did not alter cerebral blood flow before injury, indicating intact cerebral autoregulation, regional cerebral blood flow fell to lower levels during hypotension than during normotension for at least 72 h post-FPI, again resolving within 168 h in the newborn pig. In contrast, cerebral blood flow fell to lower levels during hypotension in all regions only at 1 h post-FPI in the juvenile pig. At 4 h post-FPI all regions had flow no different during hypotension than during normotension except for the cerebrum, which, in turn, returned to equivalent flows during hypotension and normotension within 8 h post-FPI. Because both pial dilation and maintenance of blood flow were impaired with a similar time course, these data strengthen the contention that there are age-related differences in the impairment of the cerebrovascular response to hypotension after FPI. It should be noted that impaired maintenance of blood flow occurred bilaterally in newborn and juvenile pigs and that such impaired maintenance of flow occurred fairly uniformly throughout the brain regions investigated in the newborn pig. Only in the juvenile pig at 4 h post-FPI was a difference in regionality detected (cerebrum affected, other regions not). These results support those of an earlier study in the adult cat in which it was observed, with the use of a weight drop model, that autoregulation is better preserved in the brain stem and posterior brain regions than in the cerebral hemispheres after brain injury (16). Finally, similar to the
et al. (19) reported that hypoperfusion was less severe after focal injury in immature than in adult rats.

In the newborn pig, FPI results in decreased pial artery diameter, reductions in cerebral blood flow and cerebral oxygenation, and altered responses to dilator stimuli (6, 9, 39, 40). Additionally, neurohumoral control of the cerebral circulation is altered after brain injury. For example, pial dilation and associated elevations in cortical periarachnoid CSF cGMP in response to several nitric oxide (NO)-dependent stimuli were attenuated after FPI in piglets (6, 40). Dilation to the NO releaser sodium nitroprusside (SNP) and the cGMP analog 8-bromo-cGMP (8-BrcGMP) appears to be dependent on activation of the K\textsubscript{ATP} channel (7). Recently, it was observed (5) that responses to SNP, 8-BrcGMP, and the K\textsubscript{ATP}-channel agonist cromakalim were blunted after FPI, suggesting that impaired function of mechanisms distal to NO synthase contributes to altered cerebral hemodynamics after FPI. Because an antagonist for the peptide ET-1, BQ-123, and the protein kinase C (PKC) inhibitor, staurosporine, partially restored decremented dilation to K\textsubscript{ATP} agonists, NO releasers, and a cGMP analog after FPI, ET-1 appears to contribute to altered cerebral hemodynamics after FPI through impairment of K\textsubscript{ATP}-channel function via activation of PKC (21). In separate experiments under non-brain injury conditions, it was also observed that coadministration of ET-1 with K\textsubscript{ATP}-channel agonists blunted dilation to such stimuli, suggesting that this peptide interacts with and impairs K\textsubscript{ATP}-channel activity (21), consistent with other studies (29).

Superoxide anion (O\textsubscript{2}•) production may contribute to altered cerebral hemodynamics after FPI because O\textsubscript{2}• scavengers partially restored decreased dilator responses after FPI (39). Moreover, ET-1, in concentrations present in CSF after FPI, has been observed to release O\textsubscript{2}• (20). ET-1 is known to activate PKC (30), whereas PKC activation results in the generation of O\textsubscript{2}• in vitro (36). Recent studies have shown that PKC activation increases O\textsubscript{2}• production on the cerebral cortical surface and contributes to such production observed after FPI in the newborn pig (2). Data from that study also showed that an activated oxygen generating system that generates an amount of O\textsubscript{2}• similar to that observed with FPI blunted pial artery dilation to K\textsubscript{ATP}-channel agonists (2). Those data, therefore, suggest that O\textsubscript{2}• generation links PKC activation to impaired K\textsubscript{ATP}-channel function after FPI. Interestingly, it has been observed that superoxide dismutase prevented the loss of compensatory vasodilation to hypotension that occurs after FPI in the adult rat (14). These data suggest that O\textsubscript{2}• contributes to impaired cerebrovascular responses to hypotension after FPI. Accordingly, it is speculated that ET-1-induced generation of O\textsubscript{2}• via PKC activation produces impairment of K\textsubscript{ATP}-channel function, which results in the observed altered cerebrovascular response to hypotension after FPI. Whereas K\textsubscript{Ca} in addition to K\textsubscript{ATP}-channel activation contributes to hypotensive pial artery dilation (3), it is presently uncertain whether ET-1 also impairs K\textsubscript{Ca}-channel function to result in the observed altered hypotensive cerebrovascular response.

In conclusion, results of the present study show that FPI disturbs cerebral autoregulation during hypotension both to a greater magnitude and for a longer duration in the newborn than in the juvenile. Although ET-1 contributes to FPI-associated disturbed autoregulation in both age groups, these data suggest that the greater FPI-induced release in the newborn could contribute to age-dependent differences in hypotensive cerebral autoregulation after FPI.

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