NOC/oFQ contributes to age-dependent impairment of NMDA-induced cerebrovasodilation after brain injury

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Received 7 February 2000; accepted in final form 23 May 2000

Armstead, William M. NOC/oFQ contributes to age-dependent impairment of NMDA-induced cerebrovasodilation after brain injury. Am J Physiol Heart Circ Physiol 279: H2188–H2195, 2000.—This study characterized the effects of fluid percussion brain injury (FPI) on N-methyl-D-aspartate (NMDA)-induced vasodilation and determined the role of nociceptin/orphanin FQ (NOC/oFQ) in such changes as a function of age and time postinsult. FPI elevated cerebrospinal fluid (CSF) NOC/oFQ from 70 ± 3 to 444 ± 56 pg/ml (∼10−10 M) within 1 h and to 1,931 ± 122 pg/ml within 8 h, whereas values returned to control levels within 168 h in the newborn pig. In contrast, FPI elevated CSF NOC/oFQ from 77 ± 4 to 292 ± 16 pg/ml within 1 h and values returned to control levels within 8 h in the juvenile pig. Topical NOC/oFQ (10−6 M) had no effect on pial artery diameter but attenuated NMDA (10−8, 10−6 M)-induced dilation (9 ± 1 and 16 ± 1 vs. 5 ± 1 and 10 ± 1%) in both age groups. In the newborn, NMDA-induced pial artery dilation was reversed to vasoconstriction within 1 h post-FPI and responses remained impaired for 72 h, but such vasoconstriction was attenuated by pretreatment with [F/G]NOC/oFQ(1–13)-NH2 (10−6 M, 1 mg/kg iv), an NOC/oFQ antagonist (9 ± 1 and 16 ± 1 vs. −7 ± 1 and −12 ± 1 vs. −2 ± 1 and −3 ± 1% for control, FPI, and FPI pretreated with the NOC/oFQ antagonist). In contrast, in the juvenile, NMDA-induced vasodilation was only attenuated within 1 h post-FPI and returned to control within 8 h. Such dilation was also partially restored by the NOC/oFQ antagonist. These data indicate that NOC/oFQ contributes to impaired NMDA pial artery dilation after FPI. These data suggest that the greater NOC/oFQ release in the newborn versus the juvenile may contribute to age-related differences in FPI effects on excitatory amino acid-induced pial dilation.

Newborn; cerebral circulation; opioids; excitatory amino acids

TRAUMATIC BRAIN INJURY is one of the major causes of morbidity, mortality, and pediatric intensive care unit admissions of children today (30, 32). Although the effects of traumatic brain injury have been well described for adult animal models (13, 22, 23, 35), few have investigated these 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 after diffuse traumatic brain injury using a weight drop model in the immature rat. Similarly, Smith et al. (31) 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 (22, 23). More recently, Prins et al. (28) 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 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 (7). Moreover, systemic arterial blood pressure increases in the juvenile pig after brain injury, consistent with other adult studies (35), whereas it decreases in the newborn pig (7). 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 mechanisms for such age-related differences post-FPI.

Glutamate is an important excitatory amino acid transmitter in the brain. It can bind to any of three different ionotropic receptor subtypes named after specific synthetic analogs: N-methyl-D-aspartate (NMDA), kainate, and AMPA. Activation of NMDA receptors has been observed to elicit cerebrovascular dilation and may represent one of the mechanisms for the coupling of local cerebral metabolism to blood flow (16). NMDA-induced pial artery dilation has been observed to be attenuated after ischemia-reperfusion in the piglet (9–11, 33). Mechanisms for such altered dilation to NMDA after such an insult have been less well characterized. Additionally, although activation of the NMDA receptor is thought to contribute to altered cerebrovascular regulation after traumatic brain injury (21), the effects of such injury on the vascular action of NMDA have been less well appreciated.

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Opioids have been observed to be important in the control of the cerebral circulation of the piglet during physiological and pathological conditions (6). During the last 5 years, several groups have isolated and cloned a new G protein-coupled receptor that showed high homology with opioid receptors (12, 17, 26). The peptide ligand for this receptor does not bind to classical opioid receptors (μ, δ, κ) and was named orphanin FQ by Reinscheid et al. (29) because its sequence begins with phenylalanine (F) and ends with a glutamine (Q). The same peptide was called nociceptin by Meunier et al. (25) because it increased the reactivity to pain in animals in contrast with the analgesic effects of opioid drugs. Recently, nociceptin/orphanin FQ (NOC/oFQ) has been observed to elicit pial artery vasodilation in the newborn pig (4). However, nothing is known about the role of NOC/oFQ in the physiological or pathophysiological control of cerebral hemodynamics. Although somewhat controversial (18, 19), the identification of a NOC/oFQ receptor antagonist, [F/G]NOC/oFQ(1–13)-NH2, and its demonstrated selectivity for NOC/oFQ in the piglet cerebral circulation (4) has resulted in the development of an avenue for the characterization of the functional significance of this newly described opioid. Interestingly, it was previously observed that NOC/oFQ can both inhibit the release of glutamate from rat cerebrocortical slices and inhibit glutamatergic transmission in the rat spinal cord as well as have its own signaling modulated by NMDA (15, 27, 36).

This study hypothesized that excitatory amino acid-induced dilation would be impaired by FPI and that NOC/oFQ would contribute to such impairment. Therefore, the present study was designed to 1) characterize the influence of FPI on cerebrospinal fluid (CSF) NOC/oFQ concentration as a function of age and time postinsult, 2) determine the effect of NOC/oFQ in a concentration similar to that observed after FPI on NMDA and glutamate-induced pial artery vasodilation, and 3) determine the functional significance of such a relationship by characterizing the effects of FPI on NMDA- and glutamate-induced vasodilation as a function of age and time postinsult in the absence and presence of the NOC/oFQ receptor antagonist [F/G]NOC/oFQ(1–13)-NH2.

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 sedated with isoflurane (1–2 mean alveolar concentration). Anesthesia was maintained with α-chloralose (30–50 mg/kg, supplemented with 5 mg/kg iv each hour). 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 ports 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 MgCl2, 1.5 CaCl2, 132 NaCl, 6.6 urea, 3.7 dextrose, and 24.6 NaHCO3. This artificial CSF was warmed to 37°C and had the following chemistry: pH 7.33, Pco2 46 mmHg, and Po2 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 microcalorimeter.

Methods for brain FPI have been described previously (35). A device designed by the Medical College of Virginia was used. A small opening was made in the parietal skull contiguously to the cranial window. A metal shaft was inserted 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 were performed 72 or 168 h post-FPI.

Protocol. Two types of pial arterial vessels, small arteries (resting diameter 120–160 μm), and arterioles (resting diameter 50–70 μm) were examined to determine whether segmental differences in the effects of FPI on NMDA and glutamate pial dilation could be identified. Pial arterial vessel diameter was determined every minute for a 1-min exposure period after infusion onto the exposed pial cortex of artificial CSF before NMDA and after the topical application of NMDA. 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 = 7), 2) newborn chronic FPI (72 h postinsult, n = 7), 3) newborn chronic FPI (168 h postinsult, n = 7), 4) newborn [F/G]NOC/oFQ(1–13)-NH2-

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pretreated acute FPI (≤8 h, n = 7), 5 newborn [F/G]NOC/oFQ-(1–13)-NH₂-pre-treated chronic FPI (72 h postinsult, n = 7), 6 newborn pretreated [F/G]NOC/oFQ-(1–13)-NH₂ chronic FPI (168 h postinsult, n = 7), 7) juvenile acute FPI (≤8 h postinsult, n = 7), 8) juvenile [F/G] NOC/oFQ-(1≤13) NH₂-pre-treated acute FPI (≤8 h postinsult, n = 7), 9) time control newborn acute FPI (8 h, n = 7), 10) time control newborn chronic FPI (72 h, n = 7), 11) time control newborn chronic FPI (168 h, n = 7), and 12) time control juvenile acute FPI (8 h, n = 7). Small arteries and arterioles were observed in each of these experiments. Topical NMDA and glutamate (10⁻⁶ M topical, 1 mg/kg iv) was administered 20 min before FPI. Confirmation of NOC/oFQ receptor blockade was determined by comparing responses before and after [F/G]NOC/oFQ(1–13)-NH₂ administration. In a separate group of animals, the interaction between NOC/oFQ and the excitatory amino acids was noted by coadministering a concentration of NOC/oFQ observed in CSF after FPI (10⁻¹⁰ M) with NMDA or glutamate and comparing such vascular responses to that observed in the absence of NOC/oFQ. The vehicle for all agents was 0.9% saline.

NOC/oFQ analysis. The CSF samples that were collected were acidified, rapidly frozen, and stored at −20°C. Radioimmunoassay kits for NOC/oFQ are commercially available (Phoenix). The radioimmunoassay uses simultaneous addition of sample, rabbit anti-NOC/oFQ antibody, and the 125I-labeled derivative of NOC/oFQ. After an overnight incubation at 4°C, free NOC/oFQ was separated from NOC/oFQ bound to antibody by the addition of goat anti-rabbit IgG serum and normal rabbit serum. After being centrifuged at 760 g for 10 min, the supernatant was decanted and the pellet was counted using a gamma scintillation counter. All samples 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 NOC/oFQ values were normally distributed and were analyzed using ANOVA for repeated measures or t-test where appropriate. 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, while the power value was 0.84. Values are represented as means ± SE of the absolute values or percent changes from control values.

RESULTS

Influence of FPI on CSF NOC/oFQ concentration in the newborn pig. Experiments were initially designed to characterize the influence of FPI on CSF NOC/oFQ concentration. Cortical periarachnoid CSF NOC/oFQ was elevated within 1 h of FPI (2.0 ± 0.1 atm) in the newborn pig (Fig. 1A). CSF NOC/oFQ concentration continued to rise and peaked at 8 h but was no different from control value within 168 h after FPI (Fig. 1A). On a molar basis, CSF NOC/oFQ was ≈10⁻¹¹ M under resting sham-treated control conditions, ≈10⁻¹⁰ M at 1 h, and ≈10⁻⁹ M at 8 h post-FPI.

Interaction between NOC/oFQ and the excitatory amino acids NMDA and glutamate. Topical NMDA and glutamate (10⁻⁸ – 10⁻⁶ M) elicited reproducible pial small artery (120–160 μm) and arteriole (50–70 μm) dilation in two different sham-treated control preparations. In the first preparation, agonist responses were obtained initially (0 h) and at 1, 4, and 8 h in an acute sham-treated control preparation (cranial window and brain injury adapter placed in the skull but no injury induced). In the second chronic survival surgery preparation (injury adapter placed in the skull and then withdrawn without injury being induced, followed by recovery and placement of window 72 or 168 h later), pial responses to NMDA and glutamate were obtained 72 or 168 h after sham surgery and compared with responses obtained initially (0 h) in the acute preparation. Responses to the agonists obtained in both such acute and chronic preparations were reproducible over time (data not shown). However, coadministration of NOC/oFQ (10⁻¹⁰ M) with either NMDA or glutamate resulted in diminished dilation to both excitatory amino acids in newborn pigs (Fig. 2A). NOC/oFQ

Fig. 1. A: influence of fluid percussion injury (FPI; 2.0 ± 0.1 atm) on cerebrospinal fluid (CSF) nociceptin/orphanin FQ (NOC/oFQ) at 1, 4, 8, 72, and 168 h post-FPI in the newborn pig. B: influence of FPI on CSF NOC/oFQ at 1, 4, and 8 h post-FPI in the juvenile pig, n = 7. *P < 0.05 compared with control (0). Please note that different value scales are used on the vertical axes in A and B.
(10^{-10} \text{ M}) by itself had no effect on pial artery diameter (141 ± 6 vs. 143 ± 7 \mu m, n = 7). Coadministered NOC/oFQ (10^{-9} \text{ M}) further diminished NMDA and glutamate pial artery dilation (data not shown).

Role of NOC/oFQ in impaired NMDA- and glutamate-induced pial artery dilation after FPI in the newborn pig. NMDA- and glutamate-induced pial small artery dilation was reversed to vasoconstriction within 1 h post-FPI (1.9 ± 0.1 atm) in the newborn (Figs. 3 and 4). Such reversal was maintained for at least 8 h, with mild vasodilation reemerging at 72 h and vasodilation being fully restored within 168 h postinsult. In animals pretreated with the NOC/oFQ antagonist [F/G]NOC/oFQ(1–13)-NH_2 (10^{-6} \text{ M}, 1 mg/kg iv) such reversal to vasoconstriction for NMDA and glutamate was attenuated at 1 h and reversed back to vasodilation at 4 h and such vasodilation was enhanced at 72 h post-FPI compared with that observed in the absence of this antagonist (Figs. 3 and 4). Pretreatment with [F/G]NOC/oFQ(1–13)-NH_2 did not alter excitatory

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**Fig. 2.** Influence of N-methyl-D-aspartate (NMDA) and glutamate (10^{-8}, 10^{-6} \text{ M}) and NMDA and/or glutamate plus coadministered with NOC/oFQ (10^{-10} \text{ M}) on pial small artery diameter in newborn (A) or juvenile (B) pigs, n = 7. *P < 0.05 compared with corresponding control.

**Fig. 3.** Influence of FPI on pial small artery dilation to NMDA (10^{-8}, 10^{-6} \text{ M}) as a function of time postinsult in newborn pigs in the absence (control) and presence of [F/G]NOC/oFQ(1–13)-NH_2 (10^{-6} \text{ M}, 1 mg/kg iv), n = 7. *P < 0.05 compared with 0. +P < 0.05 compared with absence of antagonist.

**Fig. 4.** Influence of FPI on pial small artery dilation to glutamate (10^{-8}, 10^{-6} \text{ M}) as a function of time postinsult in newborn pigs in the absence (control) and presence of [F/G]NOC/oFQ(1–13)-NH_2 (10^{-6} \text{ M}, 1 mg/kg iv), n = 7. *P < 0.05 compared with 0. +P < 0.05 compared with absence of antagonist.
amino acid pial vasodilation either before or at 168 post-FPI. Similar results were obtained in pial arterioles. Confirmation of the effectiveness of NOC/oFQ receptor blockade was performed by obtaining responses to topical NOC/oFQ at 1, 4, 8, and 72 h post-[F/G]NOC/oFQ(1–13)-NH₂ administration in FPI animals and comparing these vascular responses to those in the absence of [F/G]NOC/oFQ(1–13)-NH₂. In all cases, blockade was >85% (e.g., NOC/oFQ, 10⁻⁸ M, produced 9 ± 1% constriction in vehicle animals, whereas it produced 1 ± 1% constriction 1 h after antagonist administration). Additionally, [F/G]NOC/oFQ(1–13)-NH₂ blocked responses to NOC/oFQ in the absence of FPI. [F/G]NOC/oFQ(1–13)-NH₂ itself had no effect on pial artery diameter (138 ± 5 vs. 139 ± 6 μm, n = 7).

Role of NOC/oFQ in impaired NMDA- and glutamate-induced pial artery dilation after FPI in the juvenile pig. CSF NOC/oFQ concentration was elevated within 1 h, but such concentrations had returned to control value within 8 h after FPI in the juvenile pig (Fig. 1B). The concentration for NOC/oFQ was higher in the newborn versus the juvenile at 1 h postinsult (Fig. 1, A and B), but this concentration still roughly approximated 10⁻¹⁰ M. Coadministered NOC/oFQ (10⁻¹⁰ M) diminished NMDA and glutamate pial artery dilation (Fig. 2B). Also, in contrast to the newborn, NMDA- and glutamate-induced pial small artery dilation was not reversed to vasoconstriction but only attenuated for 4 h and was fully restored within 8 h post-FPI in the juvenile pig (Figs. 5 and 6). In animals pretreated with [F/G]NOC/oFQ(1–13)-NH₂ (10⁻⁶ M topical, 1 mg/kg iv), such attenuated excitatory amino acid dilation was partially restored (Figs. 5 and 6). Similar results were obtained for pial arterioles (data not shown). Confirmation of effective NOC/oFQ receptor blockade was made similar to that performed in the newborn (data not shown).

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 7.46 ± 0.02, 36 ± 3, and 93 ± 5 mmHg vs. 7.45 ± 0.02, 37 ± 3, and 94 ± 5 mmHg for pH, PCO₂, and PO₂, respectively, before and after injury in acute (8 h) experiments. Similar values were obtained for chronic survival surgery pigs at 72 and 168 h postinsult. Administration of the NOC/oFQ receptor antagonist did not significantly effect blood chemistry values or mean arterial blood pressure. Mean arterial blood pressures were 70 ± 5 and 54 ± 7 mmHg in newborns and 72 ± 5 and 63 ± 4 mmHg in juveniles before and after injury (8 h) in acute experiments. The amplitude of the pressure pulse, used as an index of injury intensity, was equivalent in newborn and juvenile animals (1.9 ± 0.1 vs. 2.0 ± 0.1 atm).

DISCUSSION

Results of the present study show that cortical peri-arachnoid CSF NOC/oFQ concentration was elevated...
within 1 h and peaked at 8 h but returned to control value within 168 h post-FPI in the newborn pig. In contrast, the CSF concentration for NOC/oFQ was less in the juvenile and such elevated levels were present for a shorter period of time (4 h) post-FPI. In fact, the CSF concentration of NOC/oFQ at 8 h post-FPI (≈10^{-9} M) in the newborn was one order of magnitude greater than that observed in the juvenile. At 1 h post-FPI, CSF NOC/oFQ concentration was modestly greater than 10^{-10} M in the newborn but approximately equal to 10^{-10} M in the juvenile.

Although the precise concentration at the receptor level is uncertain, coadministration of NOC/oFQ (10^{-7} M) with NMDA or glutamate diminished the pial artery dilation induced by these excitatory amino acids. Coadministration of NOC/oFQ (10^{-9} M) further diminished NMDA and glutamate pial artery dilation. These data suggest that such concentrations of this opioid observed after FPI in the newborn could have physiological significance. A second series of experiments, then, were designed to determine the functional significance of the above-noted interaction of NOC/oFQ with NMDA and glutamate. The results of these studies show that FPI reversed NMDA- and glutamate-induced pial artery dilation to vasoconstriction at 1 h postinsult in the newborn. Such reversal was maintained for at least 8 h with mild vasodilation reemerging at 72 h and vasodilation being fully restored at 168 h in the newborn. Such diminished excitatory amino acid-mediated dilation occurred concomitant with the elevation of CSF NOC/oFQ concentration. Additionally, the putative NOC/oFQ antagonist [F/G]NOC/oFQ(1–13)-NH2 partially restored decremented NMDA and glutamate pial dilation observed postinsult. Taken together, these data suggest that NOC/oFQ contributes to impaired NMDA- and glutamate-mediated pial artery dilation after FPI. Because topical [F/G]NOC/oFQ(1–13)-NH2 blocked NOC/oFQ pial artery dilation under nonischemic conditions, as reported previously (4), as well as after FPI, these data indicate that these agents are a respective agonist and antagonist in the pial vascular system. Previous studies (4) have also shown that [F/G]NOC/oFQ(1–13)-NH2 did not have any effect on the pial response to either endogenous or synthetic selective agonists for the μ-, δ1-, δ2-, or κ-opioid receptor, indicating its selectivity for the ORL-1 (NOC/oFQ) receptor. [F/G]NOC/oFQ(1–13-NH2 also did not have any effect on pial artery diameter by itself, suggesting that NOC/oFQ probably has little contribution to pial vascular tone during resting physiological conditions. In that pial small arteries exhibited about the same percentage decrease in responsiveness to NMDA and glutamate after FPI as that observed with pial arterioles, these data also suggest that there are probably minimal regional segmental vascular differences in altered excitatory amino acid activity after FPI.

In contrast, there were several differences in the observed parameters described above after FPI in the juvenile versus those after this insult in the newborn. For example, NMDA- and glutamate-mediated vasodilation was not reversed to vasoconstriction but only attenuated at 1 h post-FPI in the juvenile. Such responses were fully restored within 8 h postinsult in the juvenile compared with 168 h in the newborn. Concomitant with less excitatory amino acid vascular derangement in the juvenile there was a lessened increase in CSF NOC/oFQ concentration after FPI in the juvenile versus the newborn as described above. These data indicate that cerebrovascular control mechanisms are more greatly altered with FPI in the newborn compared with the juvenile. However, when coadministered with NMDA and glutamate under nonbrain injury conditions, the same concentration of NOC/oFQ (e.g., 10^{-9} M) elicited similar inhibition of excitatory amino acid-induced pial artery dilation in the newborn and juvenile. These data suggest that while NOC/oFQ contributes to vascular derangement in both age groups, age-related differences in the magnitude of such derangement probably primarily result from the greater CSF NOC/oFQ concentration in the newborn as opposed to an inherently greater inhibitory action of the same concentration of NOC/oFQ in newborn versus in juveniles. On the basis of interspecies extrapolation of brain growth curves (14), the age of newborn pigs chosen in the present study may approximate the newborn-to-infant time period in the human. Correspondingly, the age period of the juvenile pig chosen in the present study may correlate to that of a human child 5–8 years of age (14).

Global cerebral ischemia in a piglet model was previously observed to result in attenuated pial artery dilation to NMDA (10, 11). Results of this study extend those of others in that the present study shows that glutamate- as well as NMDA-induced pial artery dilation is altered in a model of injury distinct from previously published reports. Additionally, others had not noted a reversal of NMDA-induced dilation to vasoconstriction after global cerebral ischemia (10, 11). Furthermore, the present study is the first to consider the element of age in altered vascular responsiveness to excitatory amino acids after brain injury.

The mechanism by which NMDA-induced pial artery dilation is altered after global cerebral ischemia-reperfusion or combined hypoxia-ischemia-reperfusion is unclear at this time. Recent work by others suggests a role for oxygen free radicals and protein synthesis (9, 11, 34). In that proposed scenario, increased cyclooxygenase synthesis might account for the previously observed role for oxygen free radicals in ischemia-reperfusion associated cerebrovascular derangement (34). Alternatively, the observed beneficial action of protein synthase inhibitors might relate to the block of the production of an unidentified regulatory protein that is rapidly overexpressed after ischemia (34). Interestingly, adenosine, which is released during hypoxia, has been observed to inhibit NMDA-induced pial artery dilation when coadministered with this excitatory amino acid (10), very similar to that observed with NOC/oFQ. In those studies it was suggested that adenosine might reduce calcium entry into nerve cells and activation of nitric oxide synthase by promoting hyper-
polarization or by blocking N- and Q-type channels (10). It was further suggested that adenosine might reduce presynaptic glutamate release and thus suppress autoamplification of glutamate effects (10). Equally interesting, then, is the observation that NOC/oFQ can both inhibit the release of glutamate from rat cerebrocortical slices and inhibit glutamatergic transmission in the rat spinal cord as well as have its own signaling modulated by NMDA (15, 27, 36).

Although many actions of NOC/oFQ have been described (24), little has been published on the functional significance of such actions due to the lack of an appropriate antagonist. Recently, however, a promising candidate for such a role has been described, [F/G]NOC/oFQ(1–13)-NH$_2$ (18). However, [F/G]NOC/oFQ(1–13)-NH$_2$ has also recently been observed to function as an agonist at the NOC/oFQ receptor when administered by intracerebroventricular injection in the conscious rat (19). Fortunately, results of a recent study support its selectivity in the piglet cerebral circulation (4). However, the experimental design of the present study did not allow for the identification of the cellular site of origin for NOC/oFQ detected in cortical periarachnoid CSF. Potential cellular sites of origin include neurons, glia, vascular smooth muscle, and endothelial cells.

Although glutamate is an excitatory neurotransmitter thought to be a predominant contributor to neurotoxicity associated with traumatic brain injury (21), little attention has been paid to the functional implications of vascular abnormalities to NMDA and glutamate after such an insult. In the present study, endogenous NOC/oFQ could either function to limit vascular responses to abnormally high glutamate levels in response to FPI or, alternatively, exacerbate it. It is speculated that the latter is more plausible. Recent data show that NOC/oFQ-induced vasodilation is reversed to vasoconstriction after FPI (unpublished observations). The preadministration of the NOC/oFQ antagonist [F/G]NOC/oFQ(1–13)-NH$_2$ attenuated reductions in cerebral blood flow observed after FPI, thereby acting in a neuroprotective or vasoprotective manner (unpublished observations). Therefore, it is hypothesized that the abnormal vascular responses to glutamate and NMDA are deleterious and that FPI-accentuated release of NOC/oFQ contributes to impaired cerebral hemodynamics via modulation of vasodilation by excitatory neurotransmitters. Although results of the present study do not allow for any conclusions regarding the anatomic location (endothelium vs. vascular smooth muscle) of the dysfunction, impaired pial artery vasodilation after ischemia-reperfusion has been observed to be associated with ultrastructural alterations of the microvascular endothelium, including more numerous cytoplasmic inclusions and areas of injured mitochondria (20). However, such impaired vasodilation after ischemia or FPI is not nonspecific or pandemic, because responses to isoproterenol are unchanged after ischemia (20) and to papaverine after FPI (5).

Opioids are important contributors to the regulation of the piglet cerebral circulation (6), including brain injury (8). Results of the present study extend such studies by characterizing the contribution of the newly described opioid NOC/oFQ to altered cerebrovascular regulation observed after FPI.

Mechanisms by which NOC/oFQ might alter excitatory amino acid-induced pial artery dilation as observed in the present study are currently uncertain. A working model, however, might involve cyclooxygenase-dependent oxygen free radical generation by NOC/oFQ (2). Such free radical generation occurs after FPI and contributes to postsischemic altered cerebrovascular control (3). In a piglet hypoxic-ischemic model, oxygen free radical generation impairs NMDA-induced pial artery dilation (34). Therefore, one possible contribution to impaired excitatory amino acid vasodilation after FPI could involve such cyclooxygenase-dependent free radical generation by NOC/oFQ. More speculative, however, would be mechanisms that might couple FPI to CSF NOC/oFQ release as well as the cellular site of origin for such release.

In conclusion, results of the present study indicate that NOC/oFQ contributes to impaired excitatory amino acid pial artery dilation after FPI. These data suggest that the greater NOC/oFQ release in the newborn versus the juvenile may contribute to age-related differences in FPI-associated effects on excitatory amino acid-induced pial artery dilation.

The author thanks Miriam Kulkarni for technical assistance in the performance of the experiments. This research was supported by grants from the National Institutes of Health, the American Heart Association-PA and DE Affiliate, and the University of Pennsylvania Research Foundation.

REFERENCES


