Superoxide Generation Links Nociceptin/Orphanin FQ (NOC/oFQ) Release to Impaired N-Methyl-D-Aspartate Cerebrovasodilation After Brain Injury

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Background and Purpose—Although activation of the N-methyl-D-aspartate (NMDA) receptor is thought to contribute to altered cerebrovascular regulation after traumatic brain injury, the effects of such injury on the vascular response to NMDA itself has been less well appreciated. The newly described opioid nociceptin/orphanin FQ (NOC/oFQ) elicits pial artery dilation, at least in part, in a prostaglandin-dependent manner and is released into cerebrospinal fluid after fluid percussion brain injury (FPI). Generation of superoxide anion (O$_2^-$) occurs after FPI, and a byproduct of cyclooxygenase metabolism is the generation of O$_2^-$. This study was designed to determine whether NOC/oFQ generates O$_2^-$, which in turn could link NOC/oFQ release to impaired NMDA-induced pial artery dilation after FPI.

Methods—Injury of moderate severity (1.9 to 2.1 atm) was produced by the lateral FPI technique in anesthetized newborn pigs equipped with a closed cranial window. Superoxide dismutase–inhibitable nitroblue tetrazolium (NBT) reduction was determined as an index of O$_2^-$ generation.

Results—Under non–brain injury conditions, topical NOC/oFQ (10$^{-10}$ mol/L, the concentration present in cerebrospinal fluid after FPI) increased superoxide dismutase–inhibitable NBT reduction from 1±1 to 20±3 pmol/mm$^2$ but had no effect itself on pial artery diameter. Indomethacin (5 mg/kg IV) blunted such NBT reduction (1±1 to 6±2 pmol/mm$^2$), whereas the NOC/oFQ receptor antagonist [F/G] NOC/oFQ (1-13) NH$_2$ (10$^{-6}$ mol/L) blocked NBT reduction. [F/G] NOC/oFQ (1-13) NH$_2$ and indomethacin also blunted the NBT reduction observed after FPI (1±1 to 15±1 versus 1±1 to 4±1 versus 1±1 to 4±1 pmol/mm$^2$ for sham, NOC/oFQ antagonist, and indomethacin-treated animals, respectively). NMDA (10$^{-8}$ and 10$^{-6}$ mol/L)–induced pial artery dilation was reversed to vasoconstriction after FPI, and [F/G] NOC/oFQ (1-13) NH$_2$ attenuated such vasoconstriction (sham 9±1% and 16±1% versus FPI –7±1% and –12±1% versus FPI–[F/G] NOC/oFQ (1-13) NH$_2$–pretreated animals –2±1% and –3±1%). Indomethacin and the free radical scavengers polyethylene glycol superoxide dismutase and catalase also partially restored NMDA-induced vasodilation.

Conclusions—These data show that NOC/oFQ, in concentrations present in cerebrospinal fluid after FPI, increased O$_2^-$ production in a cyclooxygenase-dependent manner and contributes to such production after FPI. These data show that NOC/oFQ contributes to impaired NMDA-induced pial artery dilation after FPI. Therefore, these data suggest that cyclooxygenase-dependent O$_2^-$ generation links NOC/oFQ release to impaired NMDA-induced cerebrovasodilation after brain injury. (Stroke. 2000;31:1990-1996.)

Key Words: cerebral circulation ■ excitatory amino acids ■ newborn ■ opioids ■ oxygen free radicals ■ pigs

Traumatic brain injury is a leading cause of morbidity and mortality in children. Decreased cerebral blood flow has been described in children after brain injury and may contribute to the severity of sequelae. Fluid percussion injury (FPI) in animals has been suggested to model human concussive trauma. In the newborn pig, FPI results in pial artery vasoconstriction and reductions in cerebral blood flow within 10 minutes of injury. Additionally, neurohumoral control of the cerebral circulation is altered after brain injury. Because the oxygen free radical scavengers polyethylene glycol superoxide dismutase (PEG-SOD) and catalase have been observed to partially restore impaired vasodilator responses to several stimuli after

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FPI, oxygen free radicals such as superoxide anion (O$_2^-$) appear to be involved in FPI-associated hemodynamic sequelae. Additionally, cortical periarachnoid cerebrospinal fluid (CSF) opioid concentrations have been shown to increase after FPI in newborn pigs and appear to contribute to altered cerebral hemodynamics after the insult. Opioids themselves are thought to be important contributors to cerebrovascular regulation in the newborn.

Glutamate is an important excitatory amino acid transmitter in the brain. It can bind to any of 3 different inotropic

Received February 22, 2000; final revision received May 15, 2000; accepted May 18, 2000.
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receptor subtypes named after specific synthetic analogues: N-methyl-d-aspartate (NMDA), kainate, and AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid). 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.\(^{10}\) NMDA-induced pial artery dilation has been observed to be attenuated after hypoxia and ischemia/reperfusion in the piglet.\(^{11-13}\) 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,\(^{14}\) the effects of such injury on the vascular action of NMDA have been less well appreciated.

During the last 5 years, several groups have isolated and cloned a new G protein–coupled receptor that showed high homology with opioid receptors.\(^{15-17}\) The peptide ligand for this receptor does not bind to classic opioid receptors (\(\mu\), \(\delta\), and \(\kappa\)) and was named orphanin FQ by Reinscheid et al\(^{18}\) because its sequence begins with phenylalanine (F) and ends with a glutamine (Q). The same peptide was called nociceptin by Meunier et al\(^{19}\) because it increased the reactivity to pain in animals in contrast to the analgesic effects of opioid drugs. Recently, nociceptin/orphanin FQ (NOC/oFQ) has been observed to elicit pial artery vasodilation in the newborn pig at least in part by a prostaglandin-dependent mechanism.\(^{20,21}\) However, little is known about the role of NOC/oFQ in the physiological or pathophysiologic control of cerebral hemodynamics. Although somewhat controversial,\(^{22,23}\) the identification of an NOC/oFQ receptor antagonist, [F/G] NOC/oFQ (1-13) NH\(_2\), and its demonstrated selectivity for NOC/oFQ in the piglet cerebral circulation\(^{20}\) have resulted in the development of an avenue for the characterization of the functional significance of this newly described opioid. Recent studies have shown that the CSF concentration of NOC/oFQ is elevated after FPI (W.M.A., unpublished observations, 2000). Interestingly, it has also been observed that NOC/oFQ can both inhibit the release of glutamate from rat cerebrocortical slices and inhibit glutamate transmission in the rat spinal cord, as well as have its own signaling modulated by NMDA.\(^{24-26}\) Finally, because a byproduct of cyclooxygenase metabolism is the generation of \(O_2^-\), and NOC/oFQ elicits dilation in a prostaglandin-dependent manner, it is uncertain whether NOC/oFQ will cause release of \(O_2^-\).

The present study, therefore, was designed to (1) determine whether NOC/oFQ, in a concentration present in CSF after FPI, increased superoxide dismutase (SOD)–inhibitable nitroblue tetrazolium (NBT) reduction, an index of \(O_2^-\) production; (2) determine whether such NOC/oFQ-mediated NBT reduction was dependent on the cyclooxygenase pathway; (3) determine whether NOC/oFQ and the cyclooxygenase pathway contribute to \(O_2^-\) production after FPI; and (4) characterize the relationship between NOC/oFQ, the cyclooxygenase pathway, and \(O_2^-\) generation in determining the effects of FPI on NMDA-induced pial artery dilation.

**Materials and Methods**

Newborn (1 to 5 days old; weight 1.3 to 2.1 kg) pigs of either sex were used in these experiments. All protocols were approved by the Institutional Animal Care and Use Committee. Animals were sedated with isoflurane (1 to 2 minimum alveolar concentration). Anesthesia was maintained with α-chloralose (30 to 50 mg/kg, supplemented with 5 mg · kg\(^{-1}\) · h\(^{-1}\) IV). 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 animal at 37°C to 39°C.

A cranial window was placed in the parietal skull of these anesthetized animals. This window consisted of 3 parts: a stainless steel ring, a circular glass coverslip, and 3 ports consisting of 17-gauge hypodermic needles attached to 3 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 mmol/L): 3.0 KCl, 1.5 MgCl\(_2\), 1.5 CaCl\(_2\), 132 NaCl, 6.6 urea, 3.7 dextrose, and 24.6 NaHCO\(_3\). This artificial CSF was warmed to 37°C and had the following chemistry: pH 7.33, PCO\(_2\) 46 mm Hg, and PO\(_2\) 43 mm Hg, 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 microscalor.

Methods for brain FPI have been described previously.\(^{27}\) 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 the exposed 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 2 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 to 2.3 atm with a constant duration of 19 to 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.

**Protocol**

Two types of pial arterial vessels, small arteries (resting diameter 120 to 160 μm) and arterioles (resting diameter 50 to 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-minute exposure period after infusion onto the exposed parietal cortex of artificial CSF before NMDA and after the topical application of NMDA. Typically, 2 to 3 mL of CSF was flushed through the window over a 30-second 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.

Eleven major types of experiments were performed: (1) generation of \(O_2^-\) with NOC/oFQ (n=7); (2) generation of \(O_2^-\) with NOC/oFQ in the presence of indomethacin (n=7); (3) generation of \(O_2^-\) with NOC/oFQ in the presence of the NOC/oFQ receptor antagonist [F/G] NOC/oFQ (1-13) NH\(_2\) (n=7); (4) generation of \(O_2^-\) with FPI (n=7); (5) generation of \(O_2^-\) with FPI in indomethacin-pretreated animals (n=7); (6) generation of \(O_2^-\) with FPI in [F/G] NOC/oFQ (1-13) NH\(_2\)-pretreated animals (n=7); (7) vascular responses to agonists in the absence of FPI (sham control) (n=7); (8) vascular responses to agonists after FPI (n=7); (9) vascular responses to agonists after FPI in indomethacin-pretreated animals (n=7); (10) vascular responses...
to agonists after FPI in [F/G] NOC/oFQ (1-13) NH₂–pretreated animals (n=7); and (11) vascular responses with FPI in PEG-SOD and catalase (SODCAT)–pretreated animals (n=7).

In the first 3 series of experiments designed to investigate generation of O₂⁻, NOC/oFQ (10⁻¹⁰ mol/L, Phoenix Pharmaceuticals, Inc.) was applied to the cerebral cortex for 20 minutes in either the absence or presence of indomethacin (5 mg/kg IV) or [F/G] NOC/oFQ (10⁻⁷ mol/L, Phoenix). In the next 3 series of experiments, generation of O₂⁻ 1 hour after FPI was investigated in the absence and presence of indomethacin or [F/G] NOC/oFQ (1-13) NH₂. In these experiments, indomethacin or [F/G] NOC/oFQ (1-13) NH₂ was administered 20 minutes before FPI. The NOC/oFQ antagonist was kept in constant contact with the cerebral cortex for the duration of the experiment. Because the technique for measurement of O₂⁻ generation (see below) involves placement of detection solutions on the cerebral cortex for 20 minutes, such measurement in fact reflects O₂⁻ generation during the first 20-minute period 1 hour after FPI.

In the vascular experiments, responses of arterial vessels to NMDA and glutamate (10⁻³ or 10⁻⁶ mol/L, Sigma) were obtained before and 1 hour after FPI either in the absence or presence of indomethacin, [F/G] NOC/oFQ (1-13) NH₂, and SODCAT (1000 U/kg and 10 000 U/kg of PEG-SOD and catalase, respectively).

O₂⁻ Analysis
SOD-inhibitable NBT reduction was determined as an index of O₂⁻ generation, as previously described. Such reduction was determined by placing NBT (Sigma, 2.4 mmol/L) dissolved in artificial CSF under 1 window and NBT (2.5 mmol/L) and SOD (Sigma, 60 U/mL) in artificial CSF under the other window 1 hour after FPI. Because such solutions remained on the surface for 20 minutes, data are quantified as picomoles of NBT reduced for 20 minutes. Two windows were placed contralateral to the adapter for induction of FPI for these experiments.

NBT is water soluble and forms a yellow solution that is converted to nitroblue formazan, an insoluble purple precipitate, in the presence of reducing agents, eg, O₂⁻. The SOD-inhibitable NBT reduction was determined by the difference in the quantities of nitroblue formazan precipitated on the brain surface under the 2 windows. Although NBT can be reduced by a variety of agents, SOD provides specificity for the assay. Slices of the brain surface 1 mm thick under each cranial window were obtained. The slices were minced and homogenized in 1N NaOH and 0.1% sodium dodecyl sulfate solution. The supernatant was discarded, and the pellet was resuspended in 3 mL of pyridine. The formazan was dissolved in the pyridine during heating at 80°C for 1 hour. Particulate matter was removed by a second centrifugation at 10 000 g for 10 minutes. The concentration of nitroblue formazan in the supernatant was then determined spectrophotometrically at 515 nm. The nitroblue formazan on the side with NBT alone was analyzed against the background of the SOD-treated side. Freshly prepared calibration solutions were used with each set of samples and treated identically to the samples. Recovery of NBT averaged 88±4%.

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 minutes, the supernatant was decanted and the pellet counted with a gamma scintillation counter. All samples and standards were assayed in duplicate. Data are calculated as %B/B₀ versus concentration, where %B=B₀−(average cpm of sample−average cpm of nonspecific binding tube)/B₀×100 and B₀=(average cpm of total binding tube−average cpm of nonspecific binding tube), where cpm is counts per minute.

Statistical Analysis
Pial arteriolar diameter, systemic arterial pressure, amount of NBT reduced, and NOC/oFQ levels were analyzed by 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 α-level of P<0.05 was considered significant in all statistical tests. Values are represented as mean±SE of the absolute values or percent changes from control values.

Results
Influence of FPI on CSF NOC/oFQ Concentration
Experiments were initially designed to characterize the influence of FPI on CSF NOC/oFQ concentration. Cortical periarachnoid CSF NOC/oFQ was elevated from 70±3 to 444±56 pg/mL within 1 hour of FPI (n=7, 2.0±0.1 atm). On a molar basis, CSF NOC/oFQ was ∼10⁻¹¹ mol/L under resting sham control conditions and ∼10⁻¹⁰ mol/L at 1 hour after FPI.

Role of the Cyclooxygenase Pathway in NOC/oFQ-Induced O₂⁻ Generation During Non–Brain Injury and Brain Injury Conditions
Topical application of NOC/oFQ (10⁻¹⁰ mol/L, the concentration present in CSF after FPI) to the cerebral cortical surface of non–brain injured animals increased SOD-inhibitable NBT reduction (Figure 1A). Such NBT reduction by NOC/oFQ was blunted by indomethacin (5 mg/kg IV) and blocked by the NOC/oFQ receptor antagonist [F/G] NOC/oFQ (1-13) NH₂ (10⁻⁶ mol/L) (Figure 1A). Under brain injury conditions, SOD-inhibitable NBT reduction was increased 1 hour after FPI (Figure 1B). Such enhanced NBT reduction after FPI was blunted by both indomethacin and [F/G] NOC/oFQ (1-13) NH₂ (Figure 1B).

Role of NOC/oFQ, the Cyclooxygenase Pathway, and O₂⁻ Generation in Impaired Excitatory Amino Acid–Induced Pial Artery Dilation After Brain Injury
NMDA and glutamate (10⁻⁴ and 10⁻⁶ mol/L) elicited reproducible pial small-artery (120 to 160 μm) and arteriole (50 to 70 μm) vasodilation in sham control animals (data not shown). However, NMDA- and glutamate-induced vasodilation was reversed to vasoconstriction within 1 hour after FPI (2.0±0.1 atm) (Figures 2 and 3). Such posts insult excitatory amino acid–induced vasoconstriction was attenuated by [F/G] NOC/oFQ (1-13) NH₂ (10⁻⁶ mol/L) (Figures 2 and 3). Both indomethacin and SODCAT administration reversed that posts insult excitatory amino acid vasoconstriction back to vasodilation, although responses were only partially restored to control value (Figures 2 and 3).

Effect of Indomethacin [F/G] NOC/oFQ (1-13) NH₂, SODCAT, and NOC/oFQ on Pial Artery Diameter
Indomethacin produced pial artery vasoconstriction (143±5 versus 129±5 μm), whereas [F/G] NOC/oFQ (1-13) NH₂, SODCAT, and NOC/oFQ (10⁻¹⁰ mol/L) had no effect on pial artery diameter.
Blood Chemistry and Injury Intensity Level
The arterial blood gas and pH for the piglets at the beginning and end of the experiments were no different between all the experimental groups (eg, 7.45 ± 0.02, 34 ± 3, and 93 ± 6 mm Hg for pH, P O 2, and P O 2). The injury intensity level was 2.0 ± 0.1 atm.

Discussion
Results of the present study show that under non–brain injury conditions, topical administration of NOC/oFQ, in a concentration observed in CSF after FPI, results in increased SOD-inhibitable NBT reduction by newborn pig brain, indicating that O 2− was generated. Because indomethacin blunted such elevation in SOD-inhibitable NBT reduction by NOC/oFQ, these data indicate that activation of cyclooxygenase contributes to O 2− generation by this opioid. Moreover, the putative NOC/oFQ antagonist, [F/G] NOC/oFQ (1-13) NH 2, blocked such NBT reduction, indicating that NOC/oFQ generates O 2− in a selective manner. Additionally, both indomethacin and [F/G] NOC/oFQ (1-13) NH 2 blunted brain injury–induced elevated SOD-inhibitable NBT reduction. Previously, FPI has been observed to be associated with generation of O 2− on the piglet cerebral cortical surface.28 In those studies, it was also observed that FPI caused the release of endothelin-1 (ET-1) into CSF, which in turn contributed to the generation of O 2− after injury through activation of protein kinase C.28,29 Results of the present study extend the latter observations in that, taken as a whole, such results suggest that NOC/oFQ also contributes to the generation of O 2− after injury through activation of cyclooxygenase. Because the concentration of NOC/oFQ observed in CSF after FPI (10−10 mol/L) did not have any effect on pial artery diameter, such O 2− generation by NOC/oFQ appears independent of vascular contributory effect. However, concerns related to the accuracy of the NBT assay have been raised recently.30

The cerebrovascular consequences of free radical production are not fully understood. However, there is a significant amount of evidence that supports a role of oxygen radicals in brain injury. For example, brain injury in cats has been reported to cause the generation of superoxide for at least 1 hour after injury.31 In that study, the sustained dilation and abnormal responsiveness of pial arterioles observed after injury could be reversed by treatment with the free radical

Figure 1. A, Determination of SOD-inhibitable NBT reduction in newborn pig brain before (control) and after topical NOC/oFQ (10−10 mol/L), as well as after coadministered indomethacin (1 mg/kg IV) or [F/G] NOC/oFQ (1-13) NH 2 (10−6 mol/L). B, Determination of SOD-inhibitable NBT reduction in newborn pig brain before (control) and after FPI, as well as after FPI plus indomethacin (INDO) or FPI plus [F/G] NOC/oFQ (1-13) NH 2; n=7. *P<0.05 vs control. †P<0.05 vs absence of indomethacin or [F/G] NOC/oFQ (1-13) NH 2.

Figure 2. A, Influence of NMDA (10−8, 10−6 mol/L) on pial small-artery diameter before (control) and after FPI, after FPI plus [F/G] NOC/oFQ (1-13) NH 2, after FPI plus indomethacin (INDO), or after FPI plus SODCAT. B, Influence of NMDA on pial arteriole diameter before (control) and after FPI, after FPI plus [F/G] NOC/oFQ (1-13) NH 2, after FPI plus indomethacin, or after FPI plus SODCAT; n=7. *P<0.05 vs corresponding control. †P<0.05 vs corresponding response in absence of indomethacin, [F/G] NOC/oFQ (1-13) NH 2, or SODCAT.
scavengers SOD and catalase. Oxygen radicals also have been shown to increase blood-brain barrier permeability, and cause abnormal arteriolar reactivity. In addition, oxygen radical scavengers have been shown to improve vascular function and blood flow during focal ischemia in rats, which may account for the observed reductions in infarct size. Intracellular generation of superoxide or other species could alter structure and/or production of nucleotides, second messengers, receptors, and membranes, and the movement of superoxide out of the cell through anion channels could result in high concentrations of activated oxygen species at cell surfaces, including endothelium. Such oxygen species are thought to antagonize NO function and to contribute to altered cerebral hemodynamics after FPI in the piglet, because free radical scavengers partially restored decreased CSF cGMP concentration and decreased responses to NO-dependent dilator stimuli such as opioids.

The role of the systemic pressor response after FPI in altered adult cerebral hemodynamics has been investigated. For example, it was hypothesized that acute elevations of blood pressure after injury in the adult result in the release and metabolism of arachidonic acid, which would generate oxygen free radicals, causing cerebral functional abnormalities. However, in contrast to studies performed in adult and juvenile animals, there was no acute elevation in blood pressure after FPI in the newborn pig. Because the elevation in systemic blood pressure was thought to be an absolute requirement for cerebral generation of free radicals after injury, the observed decrease in blood pressure was perplexing initially. More recent studies, however, have shown that the peptide ET-1 is released after FPI in the piglet. Topical administration of ET-1 in the same concentration observed after FPI resulted in the generation of substantial amounts of superoxide on the cerebral cortical surface. These results, therefore, link the cerebral release of the peptide to superoxide generation after FPI in the piglet. Interestingly, decreased opioid-induced dilation and associated CSF cGMP release after FPI were partially restored in animals pretreated with the ET-1 antagonist BQ 123. These data, then, suggest that ET-1 contributes to altered cerebral hemodynamics after FPI at least in part through elevated superoxide production.

Because it had been observed previously that NOC/oFQ interacts with NMDA and glutamate in studies unrelated to vascular activity, additional studies were designed to investigate the relationship between NOC/oFQ, O$_2^-$, the cyclooxygenase pathway, and excitatory amino acid–induced vascular activity after FPI. Results of those studies show that NMDA- and glutamate-induced pial artery dilation was reversed to vasoconstriction after FPI. Such postinsult excitatory amino acid–induced vasoconstriction was attenuated by NOC/oFQ, O$_2^-$, and SODCAT administration reversed the postinsult excitatory amino acid vasoconstriction back to vasodilation, although responses were only partially restored to control values. Taken together, these data suggest that cyclooxygenase-dependent O$_2^-$ generation links NOC/oFQ release to impaired NMDA- and glutamate-induced pial artery dilation after brain injury. However, because both indomethacin and SODCAT restored such excitatory amino acid acid dilation to a greater extent than NOC/oFQ, O$_2^-$, those data further suggest that other yet to be determined factors also contribute to activation of cyclooxygenase, subsequent O$_2^-$ generation, and final impairment of excitatory amino acid–induced vasodilation after FPI.

Global cerebral ischemia in a piglet model has previously been observed to result in attenuated pial artery dilation to NMDA. Results of the present 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.

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. In that proposed scenario, increased cyclooxygenase synthesis might account for the previously observed role for oxygen free radicals in...
ischemia/reperfusion-associated cerebrovascular derangement. Nevertheless, the observed beneficial action of protein synthesize inhibitors might relate to the blocking of the production of an unidentified regulatory protein that is rapidly overexpressed after ischemia. Interestingly, adenosine, which is released during hypoxia, has been observed to inhibit NMDA-induced pial artery dilation when coadministered with this excitatory amino acid, very similarly 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 hyperpolarization or by blocking N- and Q-type channels. It was further suggested that adenosine might reduce presynaptic glutamate release and thus suppress autoamplification of glutamate effects. 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. More distal mechanisms by which NOC/oFQ-induced O$_2^-$ generation might alter NMDA-induced pial artery dilation as observed in the present study are currently uncertain.

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, 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 after FPI or, alternatively, exacerbate them. It is speculated that the latter is more plausible. Recent data show that at higher concentrations than that studied presently, NOC/oFQ-induced vasodilation is reversed to vasoconstriction after FPI (W.M.A., unpublished observations, 2000). The preadministration of the NOC/oFQ antagonist [F/G] NOC/oFQ (I-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.

Opioids are important contributors to the regulation of the pial cerebral circulation, including brain injury. 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.

In conclusion, results of the present study show that NOC/oFQ, in concentrations present in CSF after FPI, increased O$_2^-$ production in a cyclooxygenase-dependent manner and contributes to such production after FPI. These data also show that NOC/oFQ contributes to impaired NMDA- and glutamate-induced pial artery dilation after FPI. These data suggest, therefore, that cyclooxygenase-dependent O$_2^-$ generation links NOC/oFQ release to impaired NMDA-induced cerebrovasodilation after brain injury.

Acknowledgments

This research was supported by grants from the National Institutes of Health, the American Heart Association—Pennsylvania/Delaware Affiliate, and the University of Pennsylvania Research Foundation.

References

It is well established that cerebral vascular reactivity is altered after experimental traumatic brain injury. Many abnormalities have been described. These include alterations in autoregulation, abnormal responses to changes in CO₂ tension, and altered responses to endothelium-dependent vasodilators. Most of these abnormalities have been attributed to generation of reactive oxygen species.

In the preceding article, Kulkarni and Armstead showed that the vasodilation in response to NMDA is also abnormal after traumatic brain injury. These investigators traced this abnormality to the generation of superoxide as a result of release of the opioid nociceptin/orphanin FQ. This peptide causes vasodilation in part by generating superoxide as a by-product of increased cyclooxygenase production. These observations extend our knowledge of the mechanisms by which traumatic brain injury affects the function of the microcirculation in the brain.

**Hermes A. Kontos, MD, PhD, Guest Editor**

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doi: 10.1161/01.STR.31.8.1990

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