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Cyclooxygenase-2–dependent Superoxide Generation Contributes to Age-dependent Impairment of G Protein–mediated Cerebrovasodilation

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Background: **Previous studies have observed that activation of cyclooxygenase-2 contributes to generation of superoxide anion after fluid percussion brain injury (FPI). This study was designed to characterize the effects of FPI on the vascular activity of two activators of a pertussis toxin-sensitive G protein, mastoparan and mastoparan-7, and the role of cyclooxygenase-2–dependent superoxide anion generation in such effects as a function of age.**

Methods: **Lateral FPI was induced in anesthetized newborn (1–5-day-old) and juvenile (3–4-week-old) pigs equipped with a closed cranial window.**

Results: **Mastoparan (10^{-8}, 10^{-6} M) elicited pial artery dilation that was blunted more in newborn *versus* juvenile pigs (9 ± 1 and 16 ± 1 *vs.* 3 ± 1 and 5 ± 1%, newborn; 9 ± 1 and 15 ± 1 *vs.* 6 ± 1 and 9 ± 1%, juveniles). Similar results were observed for mastoparan-7 but the inactive analog mastoparan-17 had no effect on pial artery diameter. Indomethacin (a cyclooxygenase-1 and cyclooxygenase-2 inhibitor), NS398 (a cyclooxygenase-2 inhibitor), and polyethylene glycol superoxide dismutase and catalase (free radical scavengers) partially restored impaired mastoparan dilation after FPI in the newborn in a roughly equivalent manner but not in the juvenile (3 ± 1 and 5 ± 1 *vs.* 8 ± 1 and 13 ± 1% newborn, 6 ± 1 and 9 ± 1 *vs.* 7 ± 1 and 10 ± 1% juvenile for NS398 pretreatment).**

Conclusions: **These data show that G protein activation elicits cerebrovasodilation that is blunted following FPI in an age-dependent manner, and suggest that cyclooxygenase-2–dependent superoxide anion generation contributes to G protein activation-induced dilator impairment after the insult in an age-dependent manner.**

TRAUMATIC brain injury is one of the major causes of morbidity, mortality, and pediatric intensive care unit admissions of children today.[1,2] Although the effects of traumatic brain injury have been well described for adult animal models,[3–5] few have investigated these effects in the newborn. 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.[3,4] Earlier studies have compared the cerebral hemodynamic effects of FPI in newborn (1–5 days old) and juvenile (3–4 weeks 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.[6] More recent studies have observed that superoxide anion (O^-_2) is generated following FPI and contributes to impaired vascular responses to stimuli.[7,8] One mechanistic source for such O^-_2 generation after FPI is cyclooxygenase (COX) activation,[9] particularly the COX-2 isoform of this enzyme.[10]

Receptors regulate the function of G proteins by catalyzing the release of bound guanosine diphosphate and the binding of guanosine triphosphate. In turn, guanosine triphosphate activates the G protein, allowing it to activate effector proteins that subsequently transmit chemical signals to elicit a biologic response. Mastoparan, an amphiphilic tetra decapeptide, catalyzes nucleotide exchange on G proteins in a manner similar to that of receptors.[11–13] Like the effect of receptor agonists, pertussis toxin inhibits the effects of mastoparan on Gi/Go proteins.[11–13] Because of its close mimic of G protein receptor interaction, use of mastoparan may serve as a useful probe for the modeling of signal transduction to elicit a biologic response.

Therefore, the present study was designed to characterize the effects of FPI on the vascular activity of two activators of a pertussis toxin-sensitive G protein, mastoparan and mastoparan-7, as a function of age and the role of COX-2 and O^-_2 in such effects in newborn and juvenile pigs.

Materials and Methods

Newborn (1–5-day-old) and juvenile (3–4-week-old) pigs of either sex were used in these experiments. The Institutional Animal Care and Use Committee approved all protocols. Animals were sedated with isoflurane (1–2 minimum alveolar concentration). Anesthesia was maintained with α-chloralose (30–50 mg/kg, supplemented with 5 $mg \cdot kg^{-1} \cdot h^{-1}$, intravenously). 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 heading pad was used to maintain the animals at rectally measured temperatures of 37–39°C.

A cranial window was placed 0.5 cm from bregma and the mid-sagittal line in the parietal skull of these anesthetized animals. This window consisted of three parts:

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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 cerebrospinal fluid (CSF), of the following composition (in mM): 3.0 potassium chloride, 1.5 magnesium chloride, 1.5 calcium chloride, 132 sodium chloride, 6.6 urea, 3.7 dextrose, and 24.6 sodium bicarbonate. This artificial CSF was warmed to 37°C and had the following chemistry: pH 7.33, P_{CO_2} 46 mmHg, and P_{O_2} 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.[5] A device designed by the Department of Bioengineering Medical College of Virginia (Richmond, Virginia) was used. A small opening was made in the parietal skull contralateral to the cranial window, also 0.5 cm from begma. 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. Two brackets mounted on a platform supported the percussion device. FPI was induced by striking the piston with a 4.8-kg pendulum. The intensity of the insult (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 insult 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 120–160 μm) and arterioles (resting diameter 50–70 μm), were examined to determine whether segmental differences in the effects of FPI could be identified. Typically, 2–3 ml CSF was flushed through the through the window over a 30-s period, and excess CSF was allowed to run off through one of the needle ports.

Ten types of experiments were performed (all n = 6): (1) newborn FPI, (2) newborn FPI pretreated with the COX-1/COX-2 inhibitor indomethacin (5 mg/kg, intravenously), (3) newborn FPI pretreated with the COX-2 inhibitor NS398 (10^{-4} M), (4) newborn pretreated with polyethylene glycol superoxide dismutase and catalase (SODCAT) (1,000 and 10,000 U/kg, intravenously), (5) juvenile FPI, (6) juvenile FPI pretreated with indomethacin, (7) juvenile FPI pretreated with NS398, (8) juvenile FPI pretreated with SODCAT, (9) newborn sham control, and (10) juvenile sham control. In experiments designed to investigate the influence of FPI on vascular responses to G protein activators, mastoparan, mastoparan-7, and the inactive analogue mastoparan-17 (10^{-8}, 10^{-6} M) were topically applied before and 60 min after FPI. Indomethacin, NS398, or SODCAT was applied 30 min prior to FPI and responses after FPI were obtained in the continued presence of these agents. Sham control experiments were designed to obtain responses to agonists initially and then again 60 min later. The vehicle for all agents was 0.9% saline, which had no effect on pial artery diameter. The percent change in artery diameter values was calculated on the basis of the diameter in the control period for each drug before FPI for (control) values, whereas the diameter present in the control period before drug administration after FPI was used for post-FPI values.

Statistical Analysis

Pial arteriolar diameter and systemic arterial pressure were analyzed using analysis of variance for repeated measures. If the value was significant, the data were then analyzed by the Dunnett test. An α level of $P < 0.05$ was considered significant in all statistical tests. Values are represented as means ± SE of the absolute values or percent changes from control values.

Results

Influence of FPI on Mastoparan-induced Pial Artery Dilation

Mastoparan and mastoparan-7 (10^{-8}, 10^{-6} M) elicited reproducible pial small artery (120–160 μm) and arteriole (50–70 μm) dilation, whereas mastoparan-17 had no effect on pial artery diameter (144 ± 6 *vs.* 146 ± 7 μm for control and mastoparan-17 10^{-6} M). Mastoparan- and mastoparan-7–induced pial artery dilation was blunted by FPI in the newborn and juvenile (figs. 1 and 2). Statistical differences between corresponding post-FPI values for newborns and juveniles were observed for every case except for mastoparan or mastoparan-7 (10^{-8} M) in pial small arteries (figs. 1 and 2). Mastoparan-17 had no effect on pial artery diameter after FPI similar to that observed prior to injury (121 ± 5 *vs.* 122 ± 6 μm).

Contribution of Cyclooxygenase to Impairment of Mastoparan-induced Pial Artery Dilation

In animals pretreated with the COX-1/COX-2 inhibitor indomethacin (5 mg/kg, intravenously) or the COX-2

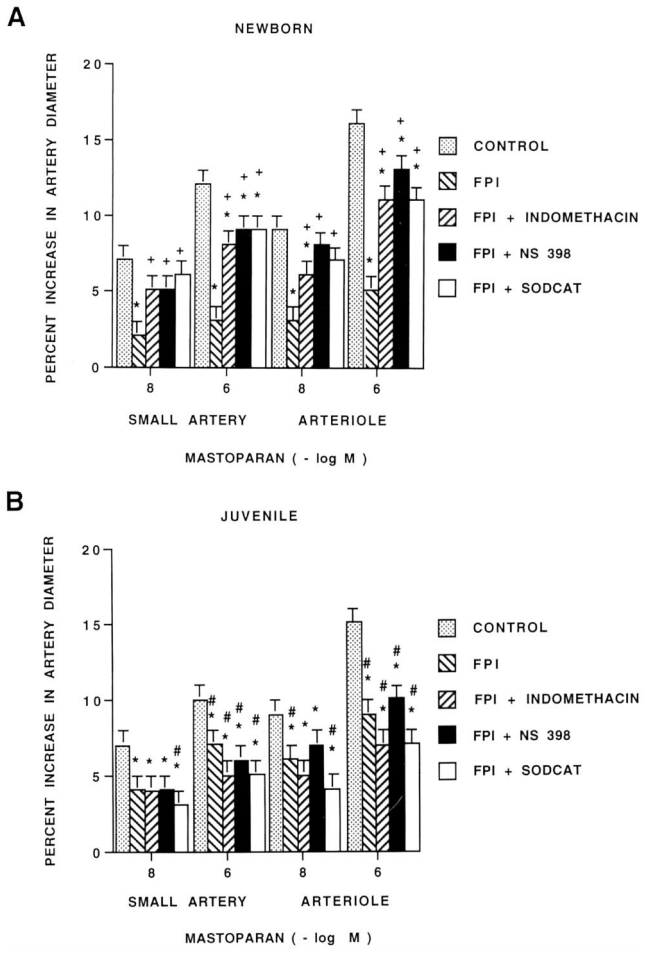

Fig. 1. (*A*) Influence of mastoparan (10^{-8}, 10^{-6} M) on pial small artery and arteriole diameter before (control), after FPI, after FPI with indomethacin (5 mg/kg, iv) pretreatment, after FPI with NS398 (10^{-4} M) pretreatment, and after FPI with SODCAT (1000 and 10,000 U/kg, iv) pretreatment in newborn pigs. (*B*) Influence of mastoparan on pial small artery and arteriole diameter before (control), after FPI, after FPI with indomethacin pretreatment, after FPI with NS398 pretreatment, and after FPI with SODCAT pretreatment in juvenile pigs (n = 6). Pial small artery and arteriole diameters were 145 ± 6 and 70 ± 4 μm at baseline and 120 ± 5 and 54 ± 4 μm after FPI. * $P < 0.05$ *versus* corresponding control value; + $P < 0.05$ *versus* corresponding nontreated FPI value; # $P < 0.05$ *versus* corresponding newborn value.

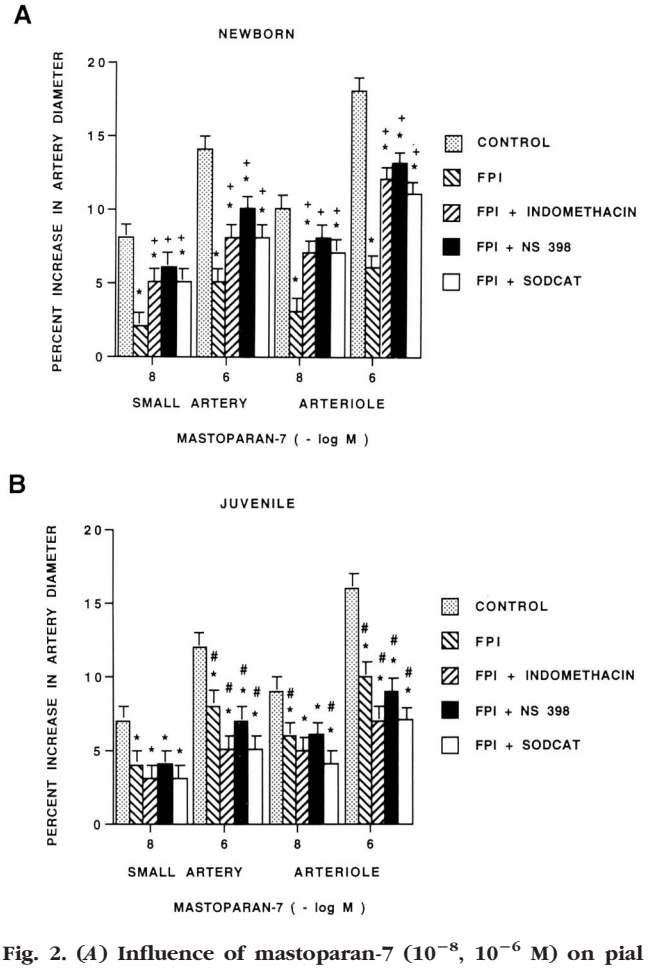

Fig. 2. (*A*) Influence of mastoparan-7 (10^{-8}, 10^{-6} M) on pial small artery and arteriole diameter before (control), after FPI, after FPI with indomethacin (5 mg/kg, iv) pretreatment, after FPI with NS398 (10^{-4} M) pretreatment, and after FPI with SODCAT (1,000 and 10,000 U/kg) pretreatment in newborn pigs. (*B*) Influence of mastoparan-7 on pial small artery and arteriole diameter before (control), after FPI, after FPI with indomethacin pretreatment, after FPI with NS398 pretreatment, and after FPI with SODCAT pretreatment in juvenile pigs (n = 6). Pial small artery and arteriole diameters were 148 ± 6 and 68 ± 5 μm at baseline and 124 ± 5 and 52 ± 4 μm after FPI. * $P < 0.05$ *versus* corresponding control; + $P < 0.05$ *versus* corresponding nontreated FPI value; # $P < 0.05$ *versus* corresponding newborn value.

inhibitor NS398 (10^{-4} M), impaired vasodilator responses to mastoparan and mastoparan-7 observed after FPI were significantly and partially restored in newborn pigs (figs. 1 and 2, *panel A*). In contrast, such impaired responses to mastoparan and mastoparan-7 post FPI were unchanged with indomethacin or NS398 pretreatment in juvenile pigs (figs. 1 and 2, *panel B*). In fact, in many instances, the resulting vasodilation to mastoparan and mastoparan-7 after FPI in such pretreated animals was significantly different between newborn and juvenile pigs (figs. 1 and 2). Indomethacin and NS398 had no effect on mastoparan- or mastoparan-7–induced pial artery dilation in the absence of FPI (131 ± 4 and 147 ± 7 μm *vs.* 119 ± 4 and 135 ± 6 μm for mastoparan before and after indomethacin).

Contribution of O^-_2 to Impairment of Mastoparan-induced Pial Artery Dilation

In animals pretreated with the O^-_2 free radical scavenger SODCAT, impaired vascular responses to mastoparan and mastoparan-7 observed after FPI were partially restored in newborn pigs (figs. 1 and 2, *panel A*). However, such impaired post-FPI responses to mastoparan and mastoparan-7 were unchanged with SODCAT pretreatment in juvenile pigs (fig. 2). SODCAT had no effect on mastoparan- or mastoparan-7–induced pial artery dilation in the absence of FPI.

Effect of Indomethacin, NS398, and SODCAT on Pial Artery Diameter

Indomethacin and NS398 produced pial artery vasoconstriction (143 ± 5 *vs.* 129 ± 5 μm and 146 ± 6 *vs.* 130 ± 6 μm for indomethacin and NS398, respectively), whereas SODCAT had no effect on diameter (142 ± 6 *vs.* 141 ± 5 μm).

Blood Chemistry and Intensity of Injury

Values for pH, P_{CO_2}, and P_{O_2} were obtained at the beginning and end of all experiments. No statistical differences were observed in any of these parameters at the end *versus* the beginning of experiments, and no group differences were noted. The amplitude of the pressure pulse, an index of injury intensity, was equivalent in newborn and juvenile pigs.

Discussion

Results of the current study show that mastoparan and mastoparan-7 elicit pial artery dilation that was blunted by FPI in the newborn pig. Although such dilation was also attenuated in the juvenile pig, the magnitude of such inhibition was significantly greater in newborns *versus* juveniles. These data indicate that G protein activation-mediated vasodilation was impaired by FPI in an age-dependent manner. Because pial small arteries and arterioles were equally affected except at low agonist concentrations (*e.g.*, 10^{-8} M), these data suggest only modest regional vascular differences in brain injury effects on G protein function. Such interactions seem specific in that the inactive analog, mastoparan-17,[14] had no effect on pial artery diameter before or after FPI, thereby serving as a negative control.

Additional results of this study show that pretreatment with both the nonselective COX-1/COX-2 inhibitor and the more selective COX-2 inhibitor NS398 partially protected impaired mastoparan- and mastoparan-7–induced pial artery dilation following FPI in the newborn pig. Because there was no statistical difference in the vascular response to these substances after the insult in indomethacin and NS398 animal groupings, these data suggest, by exclusion, that activation of the COX-2 isoform is primarily responsible for such injury-associated impairment. In contrast, however, neither indomethacin nor NS398 had any significant effect on the impaired response to mastoparan or mastoparan-7 observed after FPI in the juvenile pig. These data, therefore, suggest that COX-2 activation contributes to G protein-mediated dilator impairment after FPI in an age-dependent manner. The concentration of NS398 used in the present study (*e.g.*, 10^{-4} M) has been shown to inhibit COX-2 with minimal interaction with COX-1.[15,16] Similarly, the dose of indomethacin used in this study has been previously observed to reduce cortical periarachnoid CSF prostaglandin concentrations to nondetectable levels and inhibit the conversion of exogenous arachidonic acid to prostaglandins on the cerebral surface by 90%.[17]

In a final series of experiments, the O^-_2 scavenger SODCAT similarly partially protected impaired mastoparan- and mastoparan-7–induced pial artery dilation following FPI in the newborn pig. COX-2 activation has previously been observed to contribute to O^-_2 generation after FPI.[10] Because the magnitude of the vascular response to mastoparan and mastoparan-7 was nonsignificantly different after FPI in the presence of indomethacin, NS398, or SODCAT, these data suggest that COX-2 activation primarily contributes to impairment of such stimuli after the insult *via* O^-_2 generation. In contrast, however, SODCAT had no effect on the impaired response to such stimuli in the juvenile pig. These data, therefore, suggest that reactive oxygen species produced by COX-2 contribute to G protein-mediated dilator impairment after FPI in an age-dependent manner. The dose of SODCAT used in this study has previously been observed to block O^-_2 generation following FPI in the pig.[8]

Mastoparan is attractive as a cellular probe for the signaling activities of G proteins. The regulation of G proteins by mastoparan and by receptors seems similar in many ways.[11–13] Recent data in the piglet demonstrate that pial artery dilation to mastoparan involves coupling *via* a pertussis toxin-sensitive G protein (W.M.A, unpublished observations, July 18, 2002). Because vascular responses to many dilator stimuli are impaired following FPI[7,8] and G proteins are involved in the coupling of an extracellular stimulus to an ultimate biologic response, the current data may give further insight into mechanisms involved in impaired cerebral hemodynamic control after the insult. Mastoparan and mastoparan-7 were used to broaden conclusions regarding G protein activation through the use of two structurally different agents, whereas mastoparan-17 was used as a negative control in that it had no observable vascular activity.

Prostaglandins are important in the control of piglet cerebral hemodynamics.[17] COX is an enzyme that catalyzes the conversion of arachidonic acid to prostaglandins. Two isoforms of COX have been identified: the constitutively expressed COX-1 and the rapidly inducible COX-2. Recent data indicate that the two isoforms of COX mediate different biologic functions. COX-1 is constitutively synthesized in many tissues and is thought to perform primarily "housekeeping" functions such as gastric protection, vascular homeostasis, and renal maintenance. In contrast to COX-1, COX-2 message and protein are normally undetectable in most tissues, including the brain, but can be induced by proinflammatory or mitogenic agents such as cytokines.[18] COX-1 and COX-2 seem to have distinct roles in the regulation of the cerebral circulation. For example, COX-1 participates in responses initiated at the vascular level such as endothe-

lium-dependent dilation in response in bradykinin or A23187, whereas COX-2 contributes exclusively to vascular responses initiated by neural activity.[16,19]

Although it is well known that the products of COX activity, prostaglandins, and O^-_2 are elevated in concentration following traumatic brain injury,[20–22] the relative effects of such injury on COX-1 and COX-2 expression and the functional significance of such modified expression have been less well characterized. Recent brain and spinal cord injury studies have focused on the COX-2 isoform and have observed its prolonged expression in neurons and glia after insult.[23–25] Correspondingly, administration of selective COX-2 inhibitors resulted in improved functional and behavioral deficits following spinal cord injury.[23,26] However, the role of COX-2 in the sequelae of brain injury has been questioned[27,28] and a protective role has even been suggested.[18] Alternatively, COX-1 expression also has been observed to be induced following traumatic brain injury.[29] Because of prolonged accumulation of COX-1 in lesioned areas after the insult, these authors suggested a primary role for COX-1 and not COX-2 in the pathophysiology of secondary injury.[29]

COX-2 may provide a link between excitotoxicity and lipid peroxidation following brain injury. For example, COX-2 is expressed by excitatory neurons[30] and expression of this isoform is regulated by *N*-methyl-D-aspartate in that the *N*-methyl-D-aspartate antagonist MK801 has been observed to block such expression.[31] These data suggest that activation of excitatory amino acid receptors may be required for COX-2 gene expression. Because excitatory amino acids are released following traumatic brain injury,[32] the subsequent expression of COX-2 and generation of O^-_2 as a by-product of COX activation could contribute to lipid peroxidation after the insult. Alternatively, COX-1 may also serve as a source for the generation of O^-_2 in that such generation is thought to play a role in the cerebrovasodilation to bradykinin, arachidonic acid, and the ionophore A23187.[19] In the piglet cerebral circulation, COX-2 seems to serve as the primary source for O^-_2 generation following FPI.[10]

On the basis of interspecies extrapolation of brain growth curves,[33] the age period of newborn pigs chosen in the current study may approximate the newborn-to-infant period in the human. Correspondingly, the age period for the juvenile pig chosen in the present study may correlate to that of a 5–8-yr-old child.[33] Although the amplitude of the pressure pulse, which reflects the intensity of the injury, was equivalent in newborn and juvenile pigs, how this force acts once it enters the skull may depend on differences in the composition and compliance of the newborn and juvenile brain. In addition, 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. It is speculated, however, that differential effects of FPI on mastoparan-induced pial artery dilation in the newborn and juvenile pig relate, at least in part, to differential brain injury effects on more distal signal-transduction mechanisms. For example, mastoparan pial dilation in the pig is dependent on activation of the adenosine triphosphate-sensitive K^+ channel.[34] Previously, it has been observed that pial dilation in response to activators of the adenosine triphosphate-sensitive K^+ channel is impaired after FPI in an age-dependent manner.[35] Therefore, age-dependent blunted mastoparan pial dilation could relate to an equally age-dependent impairment of adenosine triphosphate-sensitive K^+ channel function after brain injury. The functional implications of this line of reasoning relate to the well-documented impairment of cerebral autoregulation during hypotension after brain injury. Pial artery dilation in response to hypotension is dependent on activation of the adenosine triphosphate-sensitive K^+ channel,[36] whereas hypotensive autoregulatory impairment after brain injury is age-dependent.[37] Therefore, impairment of autoregulation during hypotension after brain injury could result from blunted G protein-mediated vasodilation after the insult.

In conclusion, this study was designed to characterize the effects of FPI on the vascular activity of activators of a pertussis toxin-sensitive G protein and the role of COX-2–dependent O^-_2 generation in such effects as a function of age. The results show that G protein activation elicits cerebrovasodilation that is blunted following FPI in an age-dependent manner. These data suggest that COX-2–dependent O^-_2 generation contributes to G protein activation-induced dilator impairment after the insult in an age-dependent manner.

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References

1. Rodriguez JG, Brown ST: Childhood injuries in the United States. Am J Dis Child 1990; 144:625–6
2. Tepas JJ, III, Dokler ML: Critical care of the injured child. Semin Pediatr Surg 1995; 4:120–7
3. McIntosh TK, Hayes RL, DeWitt DS, Agura V, Faden AI: Endogenous opioids may mediate secondary damage after experimental brain injury. Am J Physiol 1987; 253:E565–74
4. McIntosh TK, Vink R, Noble L, Yamakami I, Fernyak S, Soares H, Faden AI: Traumatic brain injury in the rat: Characterization of a lateral fluid percussion mode. Neuroscience 1989; 28:233–44
5. Wei EP, Dietrich WD, Povlishock JT, Navari RM, Kontos HA: Functional, morphological, and metabolic abnormalities of the cerebral microcirculation after concussive brain injury in cats. Circ Res 1980; 46:37–47
6. Armstead WM, Kurth CD: Different cerebral hemodynamic responses following fluid percussion brain injury in the newborn and juvenile pig. J Neurotrauma 1994; 11:487–97
7. Armstead WM: Influence of brain injury on vasopressin induced pial artery vasodilation: Role of superoxide anion. Am J Physiol 1996; 70:H1272–8
8. Thorogood M, Armstead WM: Influence of PEG-SOD/catalase on altered opioid-induced pial artery dilation following brain injury. ANESTHESIOLOGY 1996; 84:614–25
9. Kulkarni M, Armstead WM: Superoxide generation links nociceptin/orphanin FQ (NOC/oFQ) release to impaired N-methyl-D-aspartate cerebrovasodilation after brain injury. Stroke 2000; 31:1990–6

10. Kulkarni M, Armstead WM: Relationship between NOC/oFQ dynorphin, and COX-2 activation in impaired NMDA cerebrovasodilation after brain injury. J Neurotrauma 2002; 19:965–73

11. Higashijima T, Burnier J, Ross EM: Regulation of G_i and G_o by mastoparan, related amphiphilic peptides, and hydrophobic amines: Mechanism and structural determinants of activity. J Biol Chem 1990; 265:14176–86

12. Klinker JF, Hageluken A, Grunbaum L, Heilmann I, Nurnberg B, Harhammer R, Offermanns S, Schwaner I, Ervens J, Wenzel-Seifert K, Muller T, Seifert R: Mastoparan may activate GTP hydrolysis by G_i-proteins in HL-60 membranes indirectly through interaction with nucleoside diphosphate kinase. Biochem J 1994; 305:377–83

13. Klinker JF, Laugwitz K-L, Hageluken A, Seifert R: Activation of GTP formation and high affinity GTP hydrolysis by mastoparan in various cell membranes, G-protein activation via nucleoside diphosphate kinase, a possible general mechanism of mastoparan action. Biochem Pharmacol 1996; 51:217–23

14. Konrad RJ, Young RA, Record RD, Smith RM, Butkerait P, Manning D, Jarett L, Wolf BW: The heterotrimeric G-protein G_i is localized to the insulin secretory granules of β-cells and is involved in insulin exocytosis. J Biol Chem 1995; 270:12869–76

15. Futaki N, Takahashi S, Yokoyama M, Arai I, Higuchi S, Otomos S: NS398, a new antiinflammatory agent, selectively inhibits prostaglandin G/H synthase, cyclooxygenase 2 (COX-2) activity in vivo. Prostaglandins 1994; 47:55–59

16. Niwa K, Aranki E, Morham SG, Ross ME, Iadecola C: Cyclooxygenase-2 contributes to functional hyperemia in whisker-barrel cortex. J Neuroscience 2000; 20:763–70

17. Leffler CW, Armstead WM, Shibata M: Role of the eicosanoids in cerebral hemodynamics, Regulation of Cerebral Blood Flow. Edited by Phillis JW. Boca Raton, CRC Press, 1993, pp 297–313

18. Dash PK, Mach SA, Moore AG: Regional expression and role of cyclooxygenase-2 following experimental traumatic brain injury. J Neurotrauma 2000; 17:69–81

19. Niwa K, Haenell C, Ross ME, Iadecola C: Cyclooxygenase-1 participates in selected vasodilator responses of the cerebral circulation. Circ Res 2001; 88: 600–8

20. Ellis EF, Wright KF, Wei EP, Kontos HA: Cyclooxygenase products of arachidonic acid metabolism in cat cerebral cortex after experimental concussive brain injury. J Neurochem 1981; 37:892–6

21. Kontos HA, Povlishock JT: Oxygen radicals in brain injury. Cent Nerv Syst Trauma 1986; 3:257–63

22. Shohami E, Shapira Y, Sidi A, Cotev S: Head injury induces increased prostaglandin synthesis in rat brain. J Cereb Blood Flow Metab 1987; 7:58–63

23. Resnick DK, Graham SH, Dixon E, Marion DW: Role of cyclooxygenase 2 in acute spinal cord injury. J Neurotrauma 1998; 15:1005–13

24. Sairanen T, Ristimaki A, Karjalainen-Lindsberg MJ, Poetau A, Koste M, Lindsberg PJ: Cyclooxygenase-2 is induced globally in infarcted human brain. Ann Neurol 1998; 43:738–47

25. Strauss KI, Barbe MF, Marshall RM, Raghupathi R, Menta S, Narayan RK: Prolonged cyclooxygenase-2 induction in neurons and glia following traumatic brain injury in the rat. J Neurotrauma 2000; 17:695–711

26. Lapchak PA, Aroujo DM Song D, Zivin JA: Neuroprotection by the selective cyclooxygenase-2 inhibitor SC-236 results in improvements in behavioral deficits induced by reversible spinal cord ischemia. Stroke 2001; 32:1220–5

27. Gong C, Ennis SR, Hoff JT, Keep RF: Inducible cyclooxygenase 2 expression after experimental intracerebral hemorrhage. Brain Res 2001; 901:38–46

28. Morham SG, Langenbach R, Loftin CD, Tiano H, Vouloumanos F, Jennette JC, Mahler JF, Kluchman KD, Ledford A, Lu CA, Smithies O: Prostaglandin synthase 2 gene disruption causes severe renal pathology in the mouse. Cell 1995; 83:473–82

29. Schwab JM, Seid K, Schluesener HJ: Traumatic brain injury induced prolonged accumulation of cyclooxygenase-1 expressing microglial brain macrophages in cats. J Neurotrauma 2001; 18:881–90

30. Kaufmann WE, Worley PF, Pegg J, Bremer M, Isakson P: COX-2, a synaptically induced enzyme is expressed by excitatory neurons at postsynaptic sites in rat cerebral cortex. Proc Natl Acad Sci U S A 1996; 93:2317–21

31. Yamagata K, Andreasson KI, Kaufmann WE, Barnes CA, Worley PF: Expression of a mitogen-inducible cyclooxygenase in brain neurons: Regulation by synaptic activity and glucocorticoids. Neuron 1993; 11:371–86

32. Katayama Y, Becker DP, Tamura T, Hovda DA: Massive increases in extracellular potassium and the indiscriminate release of glutamate following concussive brain injury. J Neurosurg 1990; 73:889–900

33. Dobbing J: The later development of the brain and its vulnerability, Scientific Foundations of Pediatrics. Edited by Davis JA, Dobbing J. London, Heineman Medical, 1981, pp 744–59

34. Armstead WM: G protein activation elicits cerebrovasodilation through interaction with K_{ATP} and K_{ca} channels. Brain Res 2002; 957:369–72

35. Armstead WM: Age dependent impairment of K_{ATP} channel function following brain injury. J Neurotrauma 1999; 16:391–402

36. Armstead WM: Hypotension dilates pial arteries by K_{ATP} and K_{ca} channel activation. Brain Res 1999; 816:158–64

37. Armstead WM: Role of endothelin-1 in age dependent cerebrovascular hypotensive responses after brain injury. Am J Physiol 1999; 274:H1888-94