NOC/oFQ and NMDA Contribute to Piglet Hypoxic Ischemic Hypotensive Cerebrovasodilation Impairment

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ABSTRACT

Previous studies have observed that hypotensive pial artery dilation was blunted after hypoxia-ischemia. In unrelated studies, the opioid nociceptin/orphanin FQ (NOC/oFQ) was observed to contribute to hypoxic ischemic impairment of N-methyl-D-aspartate (NMDA)-induced pial dilation. This study determined the contribution of NOC/oFQ and NMDA to hypoxic ischemic hypotensive cerebrovasodilation impairment in newborn pigs equipped with a closed cranial window. Global cerebral ischemia was produced via elevated intracranial pressure. Hypoxia decreased Po_2 to 33 ± 3 mm Hg. Topical NOC/oFQ (10^{-10} M), the cerebrospinal fluid concentration after hypoxia-ischemia, had no effect on pial artery diameter by itself but attenuated hypotension (mean arterial blood pressure decrease of $44 \pm 2\%$) -induced pial artery dilation ($35 \pm 2\%$ *versus* $22 \pm 3\%$). Hypotensive pial artery dilation was blunted by hypoxia-ischemia, but such dilation was partially protected by pretreatment with the putative NOC/oFQ receptor antagonist, [F/G] NOC/oFQ (1–13) NH$_2$ (10^{-6} M; $29 \pm 2\%$, sham control; $7 \pm 2\%$, hypoxia-ischemia; and $13 \pm 2\%$, hypoxia-ischemia and [F/G] NOC/oFQ (1–13) NH$_2$). Coadministration of the NMDA antagonist MK801 (10^{-5} M) with NOC/oFQ(10^{-10} M) partially prevented hypotensive pial dilation impairment. Similarly, pretreatment with MK801 partially protected hypoxic ischemia impairment of hypotensive pial dilation ($35 \pm 2\%$, sham control; $7 \pm 1\%$, hypoxia-ischemia; $22 \pm 2\%$, hypoxia-ischemia + MK801). These data show that NOC/oFQ and NMDA contribute to hypoxic ischemic hypotensive cerebrovasodilation impairment. These data suggest that NOC/oFQ modulation of NMDA vascular activity also contributes to such hypotensive impairment. (***Pediatr Res*** **51: 586–591, 2002**)

Abbreviations
H/I, hypoxia-ischemia
CSF, cerebrospinal fluid
H/I/R, hypoxia-ischemia-reperfusion
NOC/oFQ, nociceptin/orphanin FQ
NMDA, N-methyl-D-aspartate

Episodes of inadequate oxygen supply to the brain can result in significant neurologic sequelae. Babies are frequently exposed to either combined or sequential hypoxia and ischemia insults during the perinatal period because of problems with delivery or respiratory management after delivery (1). One contributor to neurologic damage is thought to be cerebrovascular dysfunction. For example, the major neurologic manifestations of brain injury in the premature infant are spastic motor deficits (1). The major neuropathologic outcomes for the latter are periventricular leukomalacia and periventricular hemorrhagic infarction (1). One contributor to periventricular leukomalacia is the pressure-passive cerebral circulation, which, in turn, can result from systemic hypotension (1). One potential pathogenic factor in such white matter injury is excess extracellular glutamate, an excitatory amino acid (1). However, little attention has been paid to the functional implications of vascular abnormalities to glutamate after an H/I insult.

During the last 5 y, several groups have isolated and cloned a new G protein-coupled receptor that showed high homology with opioid receptors (2). The peptide ligand for this receptor does not bind to classic opioid receptors (μ, δ, κ) and was named orphanin FQ by Reinscheid *et al.* (3) because its sequence begins with phenylalanine (F) and ends with glutamine (Q). The same peptide was called nociceptin by Meunier *et al.* (4) because it increased the reactivity to pain in animals in contrast with the analgesic effects of opioid drugs. However, little is known about the role of NOC/oFQ in the physiologic or pathophysiologic control of cerebral hemodynamics. Recent studies have shown that the CSF concentration of NOC/oFQ is elevated to approximately 10^{-10} M after H/I in the piglet (5, 6). Interestingly, it has also been observed to contribute to the reduction in cerebral blood flow that occurs

after H/I (5), as well to impaired glutamate- and NMDA-, a subtype of glutamate receptor, induced pial artery dilation after this insult (6). Finally, NOC/oFQ has been observed to exacerbate excitotoxic white matter lesions in a murine neonatal brain injury model (7).

Previous studies using a piglet model of global cerebral ischemia have observed that the ability of pial arteries to dilate during hypotension was impaired (8). However, the mechanism for such impairment is uncertain. This study, therefore, was designed to determine the contribution of NOC/oFQ and NMDA to H/I hypotensive cerebrovasodilation impairment in the newborn pig. In particular, this study determined *1*) the ability of NOC/oFQ, in concentrations present in CSF after H/I, to modulate hypotensive pial artery dilation, *2*) the contribution of NOC/oFQ to impaired hypotensive pial dilation after H/I, *3*) the role of NMDA in NOC/oFQ modulation of hypotensive pial dilation, and *4*) the contribution of NMDA to hypotensive dilation impairment after H/I.

METHODS

Newborn (1–5 d old, 1.3–2.1 kg) pigs of either sex were used in these experiments. All protocols were approved by the Institutional Animal Care and Use Committee. Piglets were initially anesthetized with isoflurane (1–2 minimum alveolar concentration). Anesthesia was maintained with α-chloralose (30–50 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 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 $MgCl_2$, 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 microscaler. For production of cerebral ischemia, a hollow stainless-steel bolt was implanted in a small (2 mm) hole in the skull.

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 H/I could be identified. Pial arterial vessel diameter was determined every minute for a 10-min exposure period after infusion onto the exposed parietal cortex of artificial CSF before drug application and after infusion of artifical CSF containing a drug. Typically, 2–3 mL of CSF was flushed through the window for a 30-s period, and excess CSF was allowed to run off through one of the needle ports.

Techniques for induction of total cerebral ischemia in the piglet have been well documented (8, 9). Briefly, total cerebral ischemia was accomplished by infusing artificial CSF into a hollow bolt in the cranium to maintain an intracranial pressure 15 mm Hg greater than the numeric mean of systolic and diastolic arterial blood (9). Intracranial pressure was monitored via a sidearm of the cranial window. Blood flow in pial arterioles, viewed with a microscope and video monitor, stopped completely on elevation of intracranial pressure and did not resume until the pressure was lowered (9). To prevent the arterial pressure from rising inordinately (Cushing response), venous blood was withdrawn as necessary to maintain mean arterial pressure no greater than 100 mm Hg. As the cerebral ischemic response subsided, the shed blood was returned to the animal. Cerebral ischemia was maintained for 20 min. In combined H/I/R animals, in which R is reperfusion, hypoxia (Po_2, 33 ± 3 mm Hg) was produced for 10 min before ischemia by decreasing the inspired O_2 via inhalation of N_2, which was immediately followed by the total ischemia protocol as described above after concomitantly restoring ventilation to room air.

Six major types of experiments were performed (all $n = 7$): *1*) vascular responses to hypotension in the absence of H/I/R (sham control), *2*) vascular responses to hypotension in the absence and presence of coadministered NOC/oFQ, *3*) vascular responses to hypotension before and after H/I/R, *4*) vascular responses to hypotension after H/I/R in [F/G] NOC/oFQ (1–13) NH_2–pretreated animals, *5*) vascular responses to hypotension in the absence and presence of coadministered NOC/oFQ and MK801, and *6*) vascular responses to hypotension after H/I/R in MK801-pretreated animals. Two levels of hypotension (moderate and severe) designed to lower mean arterial blood pressure by approximately 25 and 45% were investigated. Hypotension was induced by rapidly withdrawing either 5–8 or 10–15 mL blood/kg. Such a drop in mean blood pressure was maintained constant for 10 min by titration of additional blood withdrawal (1–5 mL) to keep arterial blood pressure from rising and by blood reinfusion (1–5 mL) to keep pressure from falling. The percent changes in artery diameter values were calculated on the basis of the diameter measured in the control period before hypotension.

Responses to hypotension were obtained before and after 60 min of reperfusion in H/I animals in the absence and presence of either the NOC/oFQ receptor antagonist, [F/G] NOC/oFQ (1–13) NH_2 (10^{-6} M) or the NMDA antagonist, MK801 (10^{-5} M). In antagonist-pretreated animals, the respective agents were administered 30 min before H/I. Confirmation of adequate receptor blockade was determined by comparing responses to either NOC/oFQ (10^{-8}, 10^{-6} M) before and after [F/G] NOC/oFQ (1–13) NH_2 or NMDA before and after MK801.

Statistical analysis. Pial artery diameter and systemic arterial pressure were analyzed for repeated measures. If the *F*

value was significant, the data were then analyzed by Fisher's protected least significant difference. An α level of $p < 0.05$ was considered significant in all statistical tests. Values are presented as mean $\pm$ SEM of absolute values or as percentages of change from control values.

RESULTS

Interaction of NOC/oFQ with hypotension-induced pial artery dilation. Two levels of hypotension (moderate and severe) elicited reproducible graded pial small artery (120–160 μm) and arteriole (50–70 μm) dilation in sham control animals. Moderate hypotension decreased mean arterial blood pressure by $24 \pm 1\%$ whereas severe did so by $44 \pm 2\%$. Pretreatment with NOC/oFQ (10^{-10} M), the concentration observed in CSF after H/I, diminished pial dilation to both levels of hypotension (Fig. 1). NOC/oFQ (10^{-10} M) had no effect on pial artery diameter by itself (140 ± 6 *versus* 142 ± 6 μm, $n = 7$). On a percentage basis, NOC/oFQ impaired hypotensive pial small artery and arteriole dilation by $45 \pm 7\%$ and $42 \pm 6\%$ during the moderate hypotension and by $37 \pm 6\%$ and $39 \pm 5\%$ during severe hypotension.

Role of NOC/oFQ in impaired hypotension-induced pial artery dilation after H/I. Pial small artery and arteriole dilation in response to both levels of hypotension was blunted within 1 h after H/I (Fig. 2). On a percentage basis, H/I/R decreased pial small artery dilation during moderate hypotension by $77 \pm 6\%$ and by $78 \pm 7\%$ during severe hypotension. Similar inhibition was observed in pial arterioles. Pretreatment with [F/G] NOC/oFQ (1–13) NH$_2$ (10^{-6} M), an NOC/oFQ receptor antagonist, 30 min before insult partially protected reduced

hypotensive pial dilation at 1 h of reperfusion (Fig. 2). This NOC/oFQ receptor antagonist had no effect on pial diameter by itself (138 ± 5 *versus* 139 ± 6 μm, $n = 7$). On a percentage basis, H/I/R decreased pial small artery dilation in such pretreated animals by $36 \pm 9\%$ during moderate and by $34 \pm 8\%$ during severe hypotension. Similar inhibition was observed in pial arterioles. Administration of [F/G] NOC/oFQ (1–13) NH$_2$ blocked vascular responses to NOC/oFQ (10^{-8}, 10^{-6} M) to an equal extent before and after H/I.

Role of NMDA in NOC/oFQ-associated impairment of hypotensive pial artery dilation in sham and H/I animals. Coadministration of the NMDA antagonist MK801 (10^{-5} M) with NOC/oFQ (10^{-10} M) partially protected reduced hypotensive pial artery dilation compared with the response observed with NOC/oFQ alone in sham control animals (Fig. 3). On a percentage basis, NOC/oFQ impaired pial small artery dilation during moderate hypotension by $22 \pm 3\%$ in such pretreated animals. Similarly, MK801 pretreatment 30 min before H/I partially protected reduced hypotensive pial dilation at 1 h of reperfusion (Fig. 4). On a percentage basis, H/I/R impaired pial artery dilation by $21 \pm 5\%$ during moderate hypotension in MK801-pretreated animals. Such protection by MK801 was significantly greater than that observed with [F/G] NOC/oFQ (1–13) NH$_2$ described above. Similar protection was observed in pial arterioles. NMDA-induced pial artery dilation was blocked by MK801 (10^{-5} M; $16 \pm 1\%$ and $25 \pm 1\%$ *versus* $1 \pm 1\%$ and $2 \pm 1\%$ for NMDA 10^{-8}, 10^{-6} M, before and after MK801, respectively). Similar inhibition of
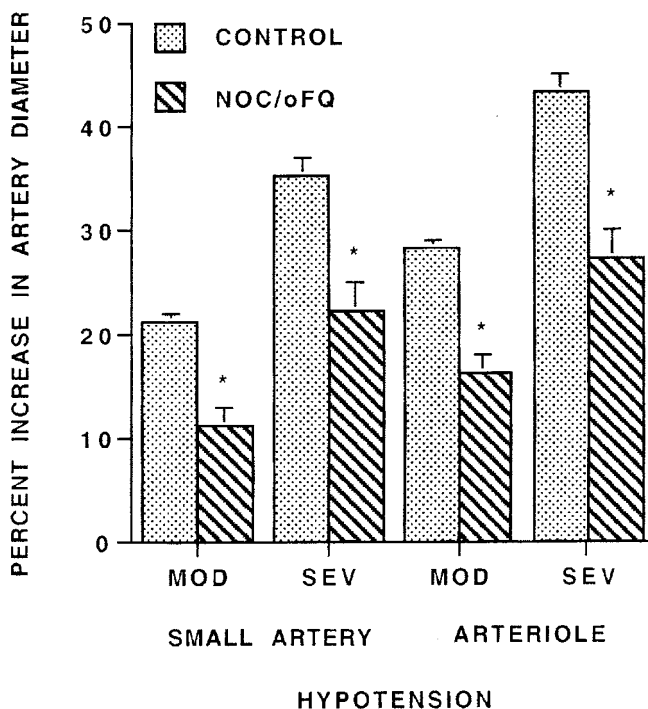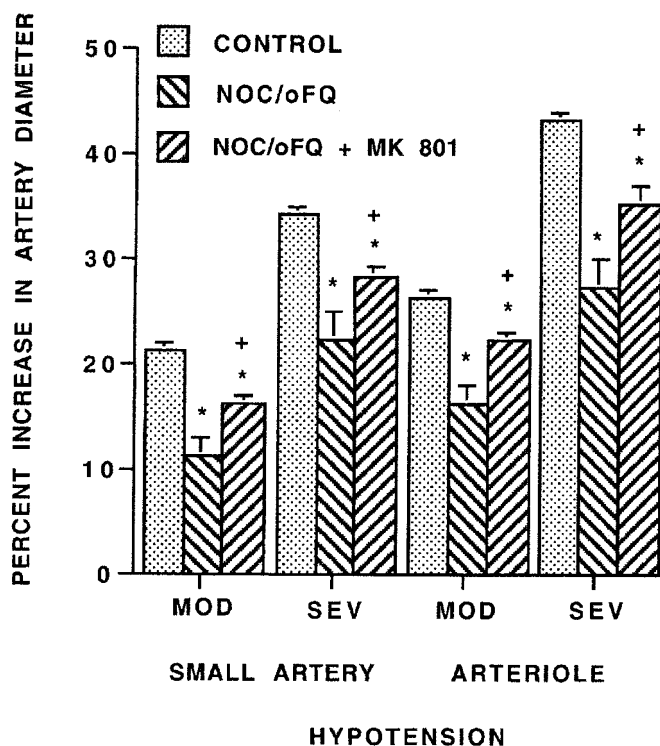

Figure 1. Influence of moderate (mod) and severe (sev) hypotension on pial small artery and arteriole diameter in the absence (control) and presence of NOC/oFQ (10^{-10} M; $n = 7$ each). *$p < 0.05$ compared with control.
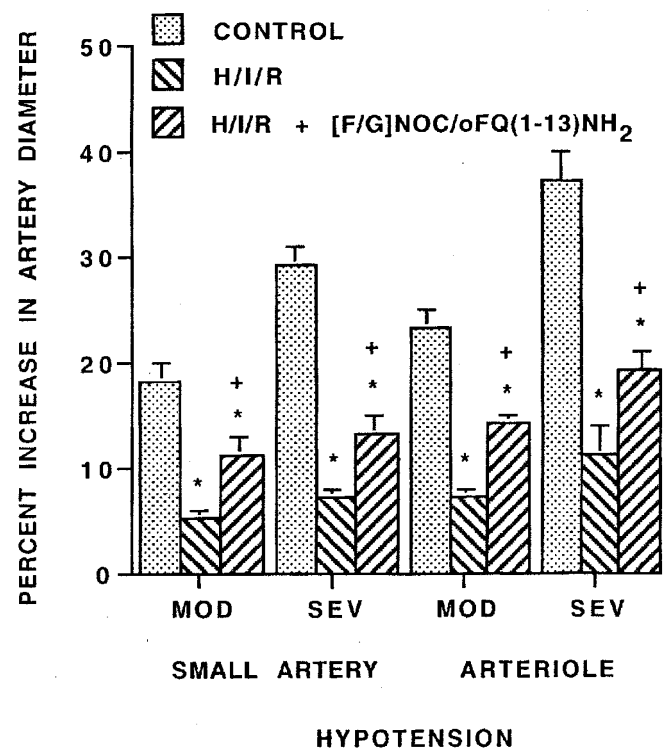
Figure 2. Influence of moderate (mod) and severe (sev) hypotension on pial small artery and arteriole diameter before (control), after H/I/R, and after H/I/R in [F/G] NOC/oFQ (1–13) NH$_2$ (10^{-6} M) -pretreated animals ($n = 7$ each). *$p < 0.05$ compared with control; +$p < 0.05$ compared with H/I/R nonpretreated value.
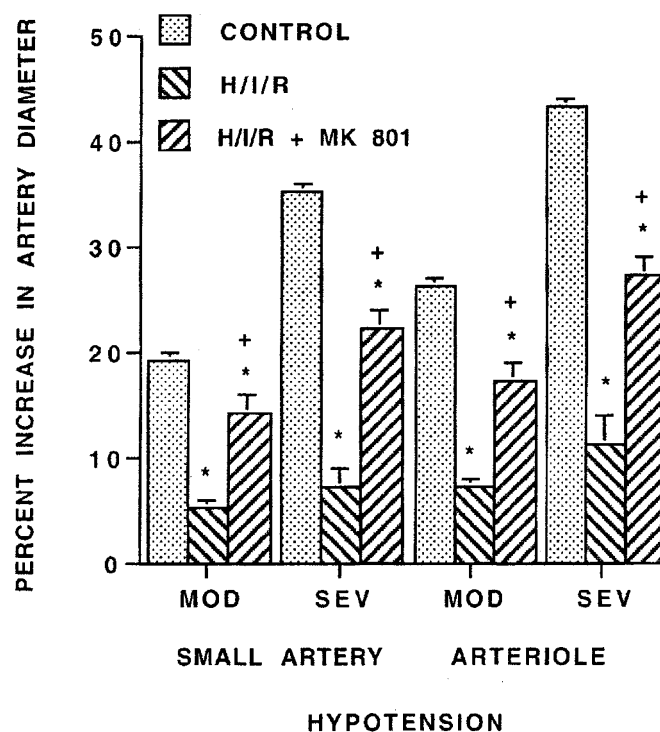
Figure 3. Influence of moderate (mod) and severe (sev) hypotension on pial artery small artery and arteriole diameter in the absence (control), presence of NOC/oFQ (10^{-10} M), and presence of coadministered NOC/oFQ and MK801 (10^{-5} M; $n = 7$ each). $*p < 0.05$ compared with control; $+p < 0.05$ compared with absence of MK801.

Figure 4. Influence of moderate (mod) and severe (sev) hypotension on pial small artery and arteriole diameter before (control), after H/I/R, and after H/I/R in MK801 (10^{-5} M) -pretreated animals ($n = 7$ each). $*p < 0.05$ compared with control; $+p < 0.05$ compared with H/I/R nonpretreated value.

Table 1. *Summary of relationships between NOC/oFQ, NMDA, and hypotensive pial artery dilation in sham and H/I animals*

	Hypotensive pial artery dilation	
	Sham	H/I
NOC/oFQ	impairs	impairs
NMDA	NOC/oFQ contributes to impairment	impairs; NOC/oFQ contributes to impairment

NMDA vascular activity by MK801 was observed after H/I. A summary of the interaction between NOC/oFQ and in impaired hypotensive pial artery dilation is shown in Table 1.

Blood chemistry. Blood chemistry and mean arterial blood pressure values were obtained at the beginning and end of all experiments as well as during hypoxia. Hypoxia decreased P_{O_2} to 33 ± 3 mm Hg, whereas the pH, P_{CO_2}, and mean arterial blood pressure values were unchanged. Values for pH, P_{CO_2}, P_{O_2}, and mean arterial blood pressure were 7.45 ± 0.02, 34 ± 4 mm Hg, 91 ± 6 mm Hg, and 68 ± 6 mm Hg, respectively, at the start of experiments *versus* 7.44 ± 0.02, 35 ± 6 mm Hg, 94 ± 7 mm Hg, and 69 ± 7 mm Hg, respectively, at the end of experiments. There were no group differences in either blood pressure or blood chemistry values.

DISCUSSION

Results of the present study show that coadministration of NOC/oFQ, in a concentration observed in cortical periarachnoid CSF after H/I (10^{-10} M) (5, 6), with hypotension attenuated pial artery vasodilation in response to this stimulus. Because this concentration of NOC/oFQ had no effect on pial artery diameter by itself, diminished hypotensive dilation did not result from physiologic antagonism. Although the precise concentration at the receptor level is uncertain, these data suggest that such concentrations of NOC/oFQ observed after H/I could have physiologic significance. Because dilation of both pial small artery and arteriole during hypotension was impaired equally, these data suggest that there are minimal

regional vascular differences in the inhibitory action of NOC/oFQ. Similarly, because both levels of hypotension-induced pial dilation were equivalently inhibited, these data indicate that such inhibition is independent of stimulus potency.

A second series of experiments was designed to determine the functional significance of the above interaction between NOC/oFQ and hypotensive pial dilation. Results of these studies show that H/I blunted hypotensive pial artery dilation at 1 h of reperfusion, similar to those previously published for cerebral ischemia without prior hypoxia (8). However, new data from this study show that the NOC/oFQ receptor antagonist [F/G] NOC/oFQ (1–13) NH$_2$ partially prevented such diminished hypotensive pial dilation after H/I. These data suggest the involvement of NOC/oFQ release after H/I in altered hypotensive dilation after this insult. Similar to results obtained with NOC/oFQ coadministered during hypotension in sham control animals, [F/G] NOC/oFQ (1–13) NH$_2$ elicited partial prevention of H/I impairment of hypotensive dilation to a roughly equal extent in both pial small arteries and arterioles during both levels of the hypotensive stimulus. Therefore, minimal regional vascular differences were observed, and such

impairment was again independent of stimulus potency. Although somewhat controversial as a selective NOC/oFQ receptor antagonist (10, 11), [F/G] NOC/oFQ (1–13) NH$_2$ has been observed to block NOC/oFQ (10^{-8}, 10^{-6} M) -induced pial artery dilation but to have no effect on the vascular response to the endogenous opioids methionine enkephalin, leucine enkephalin, dynorphin, and β endorphin or the synthetic opioids [D Ala^2,N-Me-Phe^4,Gly^5-Ol]-Enkephalin (DAMGO), [D-Pen^{2,5}]-Enkephalin (DPDPE), deltorphin, and U50,488H, μ, δ_1, δ_2, and κ opioid selective agonists (12). These data indicate that [F/G] NOC/oFQ (1–13) NH$_2$ is a selective NOC/oFQ receptor antagonist in the piglet cerebral circulation.

Additional experiments were designed to characterize the role of NMDA receptor activation in NOC/oFQ-associated impairment of hypotensive pial artery dilation in sham control and H/I animals. Results of these studies show that the NMDA receptor antagonist MK801 partially protected reduced hypotensive pial artery dilation both when coadministered with NOC/oFQ (10^{-10} M) in sham control animals and after H/I. These results suggest that NMDA receptor activation contributes to NOC/oFQ-induced impairment of hypotensive pial dilation in sham control and H/I animals. Although uncertainty exists as to the mechanism that might link NOC/oFQ to NMDA receptor activation in effecting hypotensive pial dilation impairment, one possibility could relate to the previously observed ability of NOC/oFQ to contribute to the reversal of NMDA from a vasodilator to a vasoconstrictor via superoxide generation after H/I (6, 13). In that case, NOC/oFQ-induced impairment of NMDA dilation might physiologically antagonize pial artery dilation during hypotension. However, as MK801 afforded greater protection of hypotensive dilation after H/I than [F/G] NOC/oFQ (1–13) NH$_2$, these data also suggest that NMDA receptor activation impairs hypotensive dilation by mechanisms independent of NOC/oFQ. No regional vascular or stimulus potency differences in the protective role for MK801 were observed. The concentration of MK801 used for investigation was efficacious for inhibition of NMDA receptor activation in that dilation to this agonist was blocked by MK801.

Glutamate is an important excitatory amino acid transmitter in the brain. It can bind to any of three different inotropic receptor subtypes named after specific synthetic analogs: NMDA, kainate, and α-amino-3-hydroxy-5-methylisoxazole-4-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 (14). Several studies have observed that NMDA-induced pial artery dilation was attenuated after hypoxia or global cerebral ischemia-reperfusion (15, 16).

The mechanism by which NMDA-induced pial artery dilation is altered after global cerebral I/R or H/I/R is unclear at this time. Recent work by others suggests a role for oxygen free radicals and protein synthesis (16, 17). In that proposed scenario, increased cyclooxygenase synthesis might account for the previously observed role for oxygen free radicals in I/R-associated cerebrovascular derangement (17). 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 (17). Interestingly, adenosine, which is released during hypoxia, has been observed to inhibit NMDA-induced pial artery dilation when coadministered with this excitatory amino acid (6), very similarly to the observations 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 (16). It was further suggested that adenosine might reduce presynaptic glutamate release and thus suppress autoamplification of glutamate effects (16). Equally interesting, then, is the observation that NOC/oFQ can inhibit the release of glutamate from rat cerebrocortical slices and inhibit glutamatergic transmission in the rat spinal cord (18, 19). NOC/oFQ signaling can also be modulated by NMDA (20). More distal mechanisms by which NOC/oFQ-induced O^-_2 generation might alter NMDA-induced pial artery dilation (13) 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.

Results of the present study extend those that previously observed an impairment of hypotensive pial artery dilation after global cerebral ischemia (8). Such results indicate that the NOC/oFQ and glutamate released after H/I (5, 21) contribute to cerebrovascular dysregulation after insult. Conversely, however, it has also been observed that both NOC/oFQ and [F/G] NOC/oFQ (1–13) NH$_2$ can inhibit ischemia-induced efflux of glutamate from rat cerebrocortical slices (22), resulting in uncertainty as to whether NOC/oFQ is neuroprotective or a contributor to neuropathology.

CONCLUSION

In conclusion, results of the present study show that NOC/oFQ and NMDA contribute to H/I hypotensive cerebrovasodilation impairment. These data suggests that NOC/oFQ modulation of NMDA vascular activity also contributes to such hypotensive vascular impairment.

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