# MINIREVIEW

# Role of Nociceptin/Orphanin FQ in the Physiologic and Pathologic Control of the Cerebral Circulation[1]

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**Nociceptin/orphanin FQ is a newly described member of the opioid family. Previous minireviews in this series have described the contribution of important factors, including opioids, in the regulation of the cerebral circulation during physiologic and pathologic conditions. The present review extends these initial comments to an opioid whose vascular actions have only very recently been appreciated. In particular, this review discusses the contribution of nociceptin/orphanin FQ to impaired cerebral hemodynamics after cerebral hypoxia/ischemia and traumatic brain injury.** Exp Biol Med 227:957–968, 2002

**Key words:** opioids; ischemia; brain injury.

The cerebral circulation is regulated by chemical stimuli, metabolic factors, perfusion pressure, and nerves. Two previous reviews in this series have been published. The first focused on local hormonal control of a paracrine/autocrine nature and on selected novel (vasopressinergic, opioid) and classic (sympathetic, cholinergic) neural-humoral stimuli for the control of the cerebral circulation (1). The second focused on only one of the above systems (e.g., opioids) and described the role of this system in the physiologic and pathophysiologic control of the cerebral circulation (2). The present review will further narrow the topic of the last review to limit the consideration to the contribution of a single newly described opioid, nociceptin/orphanin FQ (NOC/oFQ), to the physiologic and pathologic control of the cerebral circulation.

## General Observations

During the last several years, several groups have isolated and cloned a new G protein-coupled receptor that showed high homology with opioid receptors (3–5). This opioid-like receptor, however, displayed no affinity for opioid ligands and remained an 'orphan' until late 1995. At that time, two independent groups (6, 7) identified a 17-amino acid peptide that did not bind to the classic opioid receptors ($\mu$, $\delta$, $\kappa$) but activated the orphan receptor in a nanomolar concentration range and would therefore be considered the endogenous ligand for the orphan receptor (8). This peptide was named orphanin FQ by Reinscheid *et al.* (6) because its sequence begins with phenylalanine (F) and ends with glutamine (Q). The same peptide was called nociceptin by Meunier *et al.* (7) because it increased the reactivity to pain in animals in contrast with the analgesic effects of opioid drugs. The orphan receptor therefore is referred to as opioid receptor-like 1 (ORL-1) and its endogenous ligand, nociceptin/orphanin FQ or for nociceptin/orphanin FQ (NOC/oFQ ). *In situ* hybridization studies have demonstrated localization of ORL-1 in several regions of the central nervous system, including the cerebral cortex, thalamus, and hypothalamus (9). A similar distribution has been observed for NOC/oFQ. It has therefore been suggested that this opioid system may play a role in memory, nociception, learning, and emotion (10). Additionally, NOC/oFQ has been observed to elicit vasodilation in the systemic and hindquar-

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ter vascular beds of the adult rat (11–15). However, less is known concerning its vascular activity, its mechanism of action, and functional significance in the cerebral circulation.

## Classic Opioids and NOC/oFQ in the Physiologic Control of the Cerebral Circulation

Opioids contribute to the regulation of cerebral hemodynamics (1). Receptor binding of classical opioids has been demonstrated on cerebral microvessels (16). Enkephalin and dynorphin immunoreactivity, indicative of innervation, has been shown in large cerebral arteries of the pig and guinea pig, respectively (17, 18). Furthermore, classic opioids have been detected in cerebrospinal fluid (CSF; 19), and CSF opioid concentrations are in the vasoactive range under control conditions (20, 21).

Investigations into the ability of classic opioids to influence the cerebral circulation have resulted in conflicting data. For example, enkephalins have been observed to produce a modest dilation in isolated feline middle cerebral arteries, minimal dilation in feline pial arteries at very high doses, and no effect on canine basilar arteries (22–24). In contrast to the somewhat inconsistent results from the above studies in adult animals, topically applied opioids have prominent effects on newborn pig pial arteries *in vivo* (25). These results indicated that μ (methionine enkephalin; 25) and δ (leucine enkephalin; 25) receptor activation elicit dilation whereas ε (β endorphin; 25) receptor activation produces pial vasoconstriction (20, 21). κ (dynorphin; 25) receptor activation produces tone-dependent responses (dilation during resting tone, constriction when cerebrovascular tone is decreased; 20, 21). Cross-selectivity pharmacologic experiments with selective antagonists confirmed that these endogenous opioids interacted with their respective receptors in a specific manner (26–32). Although the mechanisms for tone-dependent responses are not known, alterations in the physical state of cell membranes that occur during dilation and constriction may play a role in the tone dependent nature of vascular responses. It is speculated that such membrane changes could result in altered receptor-effector coupling mechanisms and associated upregulation/downregulation of intracellular signaling mechanisms.

Because classic opioids had prominent vascular effects in the newborn pig, the actions of NOC/oFQ were investigated in this species as well. Results of that study show that NOC/oFQ ($10^{-8}$, $10^{-6}$M) elicited pial artery dilation (33). To characterize potential mechanisms involved in NOC/oFQ-induced pial dilation, additional studies were designed to determine the role of the second messengers cGMP and AMP, as well as opening of the ATP-sensitive $K^+$ ($K_{ATP}$) and calcium-sensitive ($K_{ca}$) channel in such dilation. NOC/oFQ ($10^{-8}$, $10^{-6}$M)-induced pial artery dilation was decreased by the protein kinase A inhibitor Rp 8 Br cAMPs and was associated with elevated CSF cAMP. Glibenclamide and iberiotoxin, $K_{ATP}$ and $K_{ca}$ channel antagonists, attenuated NOC/oFQ-induced dilation. In contrast, the nitric oxide (NO) synthase inhibitor, L-NNA and the protein kinase G inhibitor, Rp 8 Br cGMPs, had no effect on NOC/oFQ dilation whereas such dilation was not associated with a change in CSF cGMP. These data show that NOC/oFQ elicits pial artery dilation, at least in part, via cAMP, $K_{ATP}$ and $K_{ca}$ channel-dependent mechanisms (33).

The membrane potential of vascular smooth muscle is a major determinant of vascular tone and activity of potassium ($K^+$) channels is a major regulator of membrane potential (34). Activation or opening of these channels increases potassium efflux, thereby producing hyperpolarization of vascular muscle. Membrane hyperpolarization closes voltage dependent calcium channels, causing relaxation of vascular muscle (35). Several types of $K^+$ channels, including $K_{ATP}$, $K_{ca}$, delayed rectifier, and inward rectifier $K^+$ channels, have been identified. Pharmacologic studies using activators and inhibitors have additionally provided functional evidence that $K^+$ channels, especially $K_{ATP}$ and $K_{ca}$ channels, regulate tone of cerebral blood vessels *in vitro* and *in vivo* (34–37). In the piglet, activation of $K_{ATP}$ but not $K_{ca}$ channels has been observed to contribute to NO and cGMP-induced pial artery dilation whereas $K_{ca}$ channels contribute to cAMP dilation (38, 39). More recent data from the piglet show that cAMP analogue-induced dilation was attenuated by glibenclamide, indicating that $K_{ATP}$ channel activation also contributes to cAMP dilation (40). However, others do not ascribe such a role of the $K_{ATP}$ channel in NO dilation because pial response to sodium nitroprusside, an NO releaser, was unchanged by the $K_{ATP}$ channel antagonist glibenclamide (41–43). Although the reasons for such differences are uncertain, such observations could result from differences in species, age, or experimental conditions. Additionally, *in vivo* approaches to the study of ion channels may be limited in that pharmacologic probes can only serve as an indirect index of ion channel contribution to vascular responsiveness. Taken together, then, these data (33) suggest that NOC/oFQ elicits pial artery dilation in the piglet via a sequential release of cAMP and subsequent $K_{ATP}$ and $K_{ca}$ channel activation.

Previous studies have characterized the interaction of NOC/oFQ with adenylate cyclase. For example, NOC/oFQ has been observed to inhibit adenyl cyclase in neuroblastoma X glioma NG 108-15 hybrid cells as well to inhibit forskolin-induced cAMP accumulation in mouse brain homogenates (10). It was therefore somewhat surprising to observe NOC/oFQ to elevate CSF cAMP and correspondingly have a cAMP-dependent vasodilator component (33). Although the reason for such experimental difference is uncertain, a potential explanation could relate to the differing relationships with cAMP in vascular and nonvascular cells. Furthermore, there is precedent for such opposing interactions with cAMP in vascular and nonvascular tissue in that the opioids methionine enkephalin and dynorphin have been observed to inhibit adenylate cyclase in Chinese hamster ovary cells (10) but to elevate CSF cAMP, which in

turn contributes to the vasodilator response, in the piglet cerebral circulation (38).

Other experiments were designed to support the idea that NOC/oFQ interacts with a receptor distinct from that of other opioids (e.g., ORL-1). These results show that NOC/oFQ-induced pial artery dilation was unchanged by β funaltrexamine, BNTX, naltrindole, norbinaltrophimine, and naloxone (33). NOC/oFQ-induced vasodilation was, however, blocked by the putative ORL-1 selective antagonist, [F/G] NOC/oFQ (1–13) $NH_2$ (33, 44). On the other hand, [F/G] NOC/oFQ (1–13) $NH_2$ had no effect on the vascular responses to the endogenous opioids, methionine enkephalin, leucine enkephalin, dynorphin, and β endorphin. Similarly, [F/G] NOC/oFQ (1–13) $NH_2$ had no effect on the synthetic opioids DAMGO, DPDPE, deltorphin, and U50, 488H, μ, $\delta_1$, $\delta_2$, and κ selective opioid agonists in the piglet cerebral circulation. These data indicate that NOC/oFQ and [F/G] NOC/oFQ (1–13) $NH_2$ are a selective agonist and antagonist for the recently described ORL-1 receptor in the piglet pial artery vascular system. Because [F/G] NOC/oFQ (1–13) $NH_2$ had no effect on pial artery diameter when topically applied, these data suggest that NOC/oFQ may have little contribution to resting cerebrovascular tone (33). However, [F/G] NOC/oFQ (1–13) $NH_2$ has also been observed to function as an agonist at the ORL-1 receptor when administered by intracerebro ventricular injection in the conscious rat (45). Reasons for such experimental differences are uncertain.

## NOC/oFQ in the Pathophysiologic Control of the Cerebral Circulation

**Hypoxia/Ischemia.** Episodes of inadequate oxygen supply to the brain can result in significant neurological sequelae. Babies are frequently exposed to either combined or sequential hypoxia and ischemia insults during the perinatal period as a result of problems with delivery or respiratory management postdelivery (46). One contributor to neurologic damage is thought to be cerebrovascular dysfunction. Previous studies have observed that global cerebral ischemia results in reductions in pial artery diameter and cerebral blood flow as well as impaired cerebrovascular control during hypotension and hypercapnia in a newborn pig model (47–49). Less, however, is known about the cerebrovascular consequences of combined hypoxia/ischemia or of the potential mechanisms for such altered cerebral hemodynamics.

Because of the above-described selectivity of [F/G] NOC/oFQ (1–13) $NH_2$ as a NOC/oFQ receptor antagonist in the piglet cerebral circulation, an avenue for the characterization of the functional significance of this newly described opioid was developed. Initial studies used a combined biochemical/pharmacologic approach to characterize the role of NOC/oFQ in reduced CSF observed after ischemia/reperfusion (I/R) and combined hypoxia/ischemia/reperfusion (H/I/R). Results of this study show that cortical periarachnoid CSF NOC/oFQ concentration was elevated within 1 hr but returned to control value within 4 hr of reperfusion after I/R (50). Blood flow in the cerebrum was also decreased within 1 hr of reperfusion but returned to control value within 4 hr of reperfusion. Interestingly, topical NOC/oFQ-induced pial artery dilation was diminished within 1 hr of reperfusion but such dilation was not different than that observed before I/R within 4 hr of reperfusion. Systemic administration of the putative NOC/oFQ-receptor antagonist [F/G] NOC/oFQ (1–13) $NH_2$ before I/R partially restored the decremented blood flow in the cerebrum observed at 1 hr of reperfusion. Taken together, these data suggest that attenuated NOC/oFQ-mediated pial artery dilation following I/R contributes to the observed decrement in blood flow in the cerebrum after this insult. Because CSF NOC/oFQ concentration was elevated as well at 1 hr of reperfusion, these data further suggest that any such NOC/oFQ contribution to decremented cerebral blood flow would be enhanced at this time point. Because topical NOC/oFQ-mediated pial dilation was blocked by systemically administered [F/G] NOC/oFQ (1–13) $NH_2$ before ischemia as well as at 1 hr and 4 hr of reperfusion, these data indicate that systemically administered [F/G] NOC/oFQ (1–13) $NH_2$ crosses the blood-brain barrier in sufficient quantity to inhibit responses to the agonist NOC/oFQ for at least 4 hr. However, [F/G] NOC/oFQ (1–13) $NH_2$ did not affect cerebral blood flow before or at 4 h of reperfusion after ischemia, suggesting that NOC/oFQ probably has little contribution to cerebral hemodynamics during resting physiologic conditions.

In contrast, several differences in the observed parameters described above were noted when the results of the effects of I/R were compared with those occurring with combined H/I/R. For example, CSF NOC/oFQ concentration was increased to a greater extent with H/I/R than was that with I/R alone (50). Hypoxia by itself also modestly elevated CSF NOC/oFQ concentration. Second, blood flow was decreased percentage wise to a greater extent at 1 hr of reperfusion and remained depressed for a longer period of time (at least 8 hr) in H/I/R than in I/R animals. Interestingly, NOC/oFQ-induced dilation was reversed to pial artery vasoconstriction at both 1 and 4 hr of reperfusion after H/I/R. At 8 hr of reperfusion such vasoconstriction was returned to modest vasodilation while at 12 hr of reperfusion NOC/oFQ dilation was no different than that observed before the insult. Systemically administered [F/G] NOC/oFQ (1–13) $NH_2$ before the insult also partially restored the decremented blood flow in the cerebrum after H/I/R. Taken together, these data show that both I/R and H/I/R elevated CSF NOC/oFQ concentration and altered NOC/oFQ-induced vascular activity. These data suggest that such elevated CSF concentrations and altered vascular activity of NOC/oFQ could contribute to altered cerebral hemodynamics after such insults. However, although it is more understandable how reversal of NOC/oFQ from a vasodilator to a vasoconstrictor could contribute to reduced cerebral blood flow after H/I/R, it is less obvious and really uncertain how

a diminished dilation to NOC/oFQ following ischemia/reperfusion results in reduced cerebral blood flow.

However, the experimental design of the above study did not allow for the identification of the cellular site of origin for NOC/oFQ detected in cortical periarachnoid CSF. Potential cellular sources include neurons, glia, vascular smooth muscle, and endothelial cells. Interestingly, an *in vitro* model of oxidative stress using hypoxia/reoxygenation has recently been shown to induce NOC/oFQ gene expression in astrocytes (51).

*Mechanisms for Hypoxic/Ischemic Impairment of NOC/oFQ Cerebrovasodilation.* A possible mechanism for impaired NOC/oFQ dilation post H/I/R or I/R could relate to an altered stimulated release of prostaglandins by this opioid. Prostaglandins are present in cortical periarachnoid CSF in concentrations that are in the vasoactive range under resting conditions and contribute to the regulation of cerebral hemodynamics in the newborn pig (1). The predominant prostaglandins in the piglet are prostaglandin (PG) $E_2$, $PGI_2$, and thromboxane $A_2$ ($TXA_2$) (1). Basal CSF prostaglandin concentrations have been shown to increase after cerebral I/R in the piglet and gerbil (52, 53) and altered stimulated release of prostaglandins appears to contribute to impaired dilation to hypotension and hypercapnia following this insult (47–49). Interestingly, other opioids such as methionine enkephalin stimulate the release of prostaglandins and the altered release of such prostaglandins by methionine enkephalin contributes to the attenuated dilation to this opioid observed after fluid percussion brain injury (54), a mimic of shaken baby syndrome, an insult which produces cerebral ischemia (2). The results of a study designed to characterize the relationship between NOC/oFQ and prostaglandins show that NOC/oFQ modestly stimulated the release of CSF 6-Keto-$PGF_1\alpha$ and $TXB_2$, the metabolites of $PGI_2$ and $TXA_2$, in sham control animals (55). After I/R, basal CSF prostaglandin levels were elevated, consistent with previous observations (52, 53). NOC/oFQ-stimulated release of 6-Keto-$PGF_1\alpha$ was blocked 1 hr post-I/R (55). In contrast, NOC/oFQ-stimulated release of $TXB_2$ was enhanced (55). Correspondingly, the 6-Keto-$PGF_1\alpha$/$TXB_2$ ratio was decreased at 1 hr post-I/R. Within 4 hr postinsult, however, such basal and stimulated prostaglandin levels had returned to their respective sham levels. Additional data from this study show that pretreatment with the cyclooxygenase inhibitor indomethacin or the $PGH_2$/$TXA_2$ receptor antagonist, SQ 29,548, partially restored decremented post I/R NOC/oFQ dilator responses (55). Therefore, the biochemical data support and corroborate the pharmacologic data and indicate that altered NOC/oFQ dilation results, at least in part, from blunted stimulus induced release of $PGI_2$ and accentuated release of $TXA_2$ (55).

However, there were several differences in the observed parameters described above following H/I/R versus that following I/R alone. For example, basal CSF 6-Keto-$PGF_1\alpha$ and $TXB_2$ levels were elevated to a greater extent and for a longer duration in H/I/R animals (55). Additionally, concomitant with blocked stimulated 6-Keto-$PGF_1\alpha$ release, NOC/oFQ-stimulated release of $TXB_2$ was greater, resulting in a larger decrease in the 6-Keto-$PGF_1\alpha$/$TXB_2$ ratio following H/I/R (55). Such results help to explain previous observations showing that dilator responses to NOC/oFQ were reversed to vasoconstriction following H/I/R (50). Similar to I/R, indomethacin or SQ 29,548 pretreatment partially restored impaired NOC/oFQ-induced pial artery dilation following H/I/R (55). Impaired stimulus-induced pial artery dilation following global cerebral ischemia may not be a generalized phenomenon, however, in that others have observed that dilator responses to N-methyl-D-aspartate (NMDA) and hypercapnia were attenuated but those to hypoxia and adenosine unchanged in the piglet after ischemia (56, 57). Reasons for such selectivity in loss of vascular responsiveness are uncertain.

An alternative mechanism for impaired NOC/oFQ dilation post H/I could relate to altered cAMP and $K^+$ channel-dependent pathways mechanisms contributory to NOC/oFQ dilation (33), postinsult. Interestingly, it has been previously observed that $K_{ATP}$ channel function is impaired after cerebral ischemia in the piglet (58). New information from these studies show that NOC/oFQ-stimulated release of cAMP was attenuated after I/R (59). In contrast, stimulated CSF cAMP release by NOC/oFQ was blocked, if not reversed, to modest decreases in CSF cAMP during the initial 4 hr post-H/I/R, and such inability to release cAMP remained for 8 hr postinsult (59). Taken together, these data suggest that the more profound impairment of NOC/oFQ-induced pial artery dilation after H/I versus that observed after ischemia could relate to the potentiated inability of this agonist to elevate CSF cAMP concentration.

To more fully determine potential contributory mechanisms for the observed decrement in NOC/oFQ-induced pial vasodilation following H/I, the effects of such insults on the ability of cAMP analogues, an adenylate cyclase activator, and activators of the $K_{ATP}$ and $K_{Ca}$ channel to elicit vasodilation were explored. Results of these studies (59) show that pial artery dilation induced by the cAMP analogues was unchanged by I/R, consistent with the observations of others (60). In contrast, results of the present study show that H/I produces blunted pial dilation to these same cAMP analogues (59). Such results extend those of previous investigations (60) and indicate that while cAMP mediated dilation is resistant to influence by ischemia, such cyclic nucleotide vasodilation is susceptible to inhibition with a more robust insult like H/I.

Additional results of the present study (59) show that pial artery dilation in response to topical pituitary adenylate cyclase activating peptide, an activator of adenylate cyclase, were attenuated after both ischemic and H/I insults. Although uncertain as to the mechanism for diminished stimulated CSF concentrations of cAMP with NOC/oFQ after ischemia or H/I, results of the latter studies suggest that an altered activation of adenylate cyclase might contribute to such diminished stimulated cAMP levels. These results are

in contrast, however, to those observed for another adenylate cyclase activator, forskolin, whose dilation was unchanged after global cerebral ischemia in the piglet (60). Reasons for such differences are uncertain but could relate to different pools/mechanisms for adenylate cyclase activation by these two substances. Other results of the present study (59) show that cromakalim, a $K_{ATP}$ channel activator, elicited pial artery dilation that was blunted after both ischemia and H/I. With respect to ischemia alone, these data are consistent with those previously published (58). Present data, however, extend those previously published in that the effects of combined H/I on $K_{ATP}$ channel function had not been considered. The final series of experiments in this study (59) investigated the effects of ischemia and H/I on vasodilation elicited by the $K_{ca}$ channel activator, NS1619. Results of those studies show that such dilation was unchanged by ischemia, consistent with previous studies (60). However, the observation that NS1619 induced pial vasodilation was blunted after combined H/I is novel in that others had previously concluded that $K_{ca}$ channel mechanisms were resistant to impairment (60). Reasons for such impairment with H/I and not ischemia alone are currently unknown.

With respect to an understanding of mechanisms involved in impairment of NOC/oFQ-induced vasodilation after ischemia alone, then, such impairment appears related to an attenuated ability to elevate CSF cAMP concentration, at least in part, caused by impaired adenylate cyclase activation, as well as to an impairment of $K_{ATP}$ channel function. Although cAMP elicits vasodilation via $K_{ATP}$ channel activation (33), such a signal transduction linkage cannot explain impaired NOC/oFQ vasodilation because cAMP analogue dilation was intact after ischemia. Therefore, cAMP-independent contribution of $K_{ATP}$ channel activation to NOC/oFQ dilation must be involved in the observed impairment after ischemia. Alternatively, a more marked inability to elevate CSF cAMP as well as impaired adenylate cyclase activation, cAMP analogue dilation, $K_{ATP}$ and $K_{ca}$ channel activation contribute to impaired NOC/oFQ-induced vasodilation after H/I. NOC/oFQ-induced pial artery dilation is dependent on cAMP, $K_{ATP}$ and $K_{ca}$ channel-dependent mechanisms to elicit pial artery dilation (33) and interference with all of the above signal transduction pathways with H/I presumably results in the more robust alteration of the vascular response with this insult.

*Role of NOC/oFQ in Hypoxic/Ischemic Impairment of NMDA and Hypercapnic Cerebrovasodilation.* 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 analogues: NMDA, kainate, and α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (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 blow flow (61). NMDA induced pial artery dilation has been observed to be attenuated after hypoxia, I/R, or combined H/I/R in the piglet (62, 63). Mechanisms for such altered dilation to NMDA following these insults have been less well characterized.

Interestingly, it has also been 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 (64–66). Because of the latter observations, more recent studies were designed to investigate the interaction between NOC/oFQ and excitatory amino acids in the piglet cerebral circulation. Results of these studies show that in non-H/I piglets co-administration of NOC/oFQ, in a concentration observed in CSF after H/I ($10^{-10}$M), with NMDA or glutamate diminished the pial artery dilation induced by these excitatory amino acids, suggesting that such concentrations of this opioid-like peptide observed after I/R could have physiologic significance (67). This concentration of NOC/oFQ, however, did not have any significant effect on pial artery diameter by itself. 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 I/R attenuated NMDA and that glutamate induced pial artery dilation at 1 hr but not at 4 hr of reperfusion (67). 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) $NH_2$, partially restored decremented NMDA and glutamate pial dilation observed at 1 hr of reperfusion. Taken together, these data suggest that NOC/oFQ contributes to impaired NMDA and glutamate mediated pial artery dilation after I/R (67).

In contrast, there were several differences in the observed parameters described above following H/I/R versus that following I/R alone. For example, NMDA and glutamate mediated vasodilation was reversed to vasoconstriction at 1 hr and 4 hr post-H/I/R. Such responses were reversed back to modest dilation within 8 hr and fully restored within 12 hr post combined H/I/R (67). In animals pretreated with [F/G] NOC/oFQ (1–13) $NH_2$, pial constriction to NMDA and glutamate at 1 hr post combined H/I/R was attenuated, whereas modest pial dilation reappeared at 4 hr and was fully restored within 12 hr postinsult. Concomitant with greater excitatory amino acid vascular derangement was a greater CSF NOC/oFQ concentration after H/I/R. These data indicate that cerebrovascular control mechanisms are more greatly altered with combined H/I/R compared with I/R alone. These data suggest that NOC/oFQ contributes to the magnified cerebrovascular derangement that occurs during combined H/I/R.

The mechanism by which NMDA induced pial artery dilation is altered after global cerebral I/R or combined H/I/R is unclear at this time. Recent work by others suggests a role for oxygen free radicals and protein synthesis (57, 68, 69). In that proposed scenario, increased cyclooxygenase synthesis might account for the previously observed role for

oxygen free radicals in I/R-associated cerebrovascular derangement (69). 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 (69). Interestingly, adenosine, which is released during hypoxia, has been observed to inhibit NMDA-induced pial artery dilation when coadministered with this excitatory amino acid (57), 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 (57). It was further suggested that adenosine might reduce presynaptic glutamate release and thus suppress auto-amplification of glutamate effects (57). 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 as well as have its own signaling modulated by NMDA (64–66). More distal mechanisms by which NOC/oFQ might alter NMDA-induced pial artery dilation as observed in the present study are currently uncertain. One possibility, however, could relate to the observation that NOC/oFQ can generate superoxide anion ($O_2^-$) in a protein kinase C (PKC)-dependent manner and contributed to such modulation after hypoxia/ischemia (70). Furthermore, data from that study (70) suggested that PKC dependent $O_2^-$ generation by NOC/oFQ links NOC/oFQ release to impaired NMDA dilation after hypoxia/ischemia.

Although glutamate is an excitatory neurotransmitter thought to be a predominant contributor to neurotoxicity associated with hypoxic-ischemic stress (71), little attention has been paid to the functional implications of vascular abnormalities to NMDA and glutamate following such an insult. In the present study, endogenous NOC/oFQ could either function to limit vascular responses to abnormally high glutamate levels after H/I/R or, alternatively, exacerbate them. It is speculated that the latter is more plausible. Recent data show that at higher concentrations than those studied presently, NOC/oFQ induced vasodilation is reversed to vasoconstriction following hypoxia-ischemia (50). The preadministration of the NOC/oFQ antagonist, [F/G] NOC/oFQ (1–13) $NH_2$, attenuated reductions in cerebral blood flow observed following hypoxia-ischemia, thereby acting in a neuroprotective or vasoprotective manner (50). Therefore, it is hypothesized that the abnormal vascular responses to glutamate and NMDA are deleterious and that H/I accentuated release of NOC/oFQ contributes to impaired cerebral hemodynamics via modulation of vasodilation by excitatory neurotransmitters.

Similar to excitatory amino acids, carbon dioxide is a powerful physiological regulator of cerebral circulation (72). Previous studies have observed that hypercapnic pial artery dilation was blunted after global cerebral ischemia in the newborn pig (73). Although impairment of prostaglandin-associated vascular responses is thought to contribute to such altered hypercapnic dilation postischemia (73), the exact mechanism remains uncertain. Interestingly, in a similar experiment design to that described above for NMDA, coadministration of NOC/oFQ in a concentration approximate to that in CSF following H/I, during conditions of elevated $pCO_2$, blunted hypercapnic pial artery dilation (74). Again, this NOC/oFQ concentration had no significant effect on pial dilation by itself. Additionally, blunted hypercapnic pial artery dilation after H/I was partially restored by pretreatment with the NOC/oFQ receptor antagonist [F/G] NOC/oFQ (1–13) $NH_2$ (74). These data, therefore, suggest that NOC/oFQ release contributes to impaired hypercapnia induced cerebrovasodilation following H/I (74).

*Functional Significance of NOC/oFQ Contribution to Hypoxic/ischemic NMDA Cerebrovasodilation Impairment.* Episodes of inadequate oxygen supply to the brain can result in significant neurological sequelae. For example, the major neurological manifestations of brain injury in the premature infant are spastic motor deficits (75). The major neuropathology for the latter are periventricular leukomalacia and periventricular hemorrhagic infarction (75). One contributor to periventricular leukomalacia is the pressure passive cerebral circulation which, in turn, can result from systemic hypotension (75). One potential pathogenic factor in such white matter injury is excess extracellular glutamate, an excitatory amino acid (75). However, little attention has been paid to the functional implications of vascular abnormalities to glutamate after a hypoxic/ischemic insult.

Accordingly, a recent study was designed to determine the contribution of NOC/oFQ and NMDA receptor activation to hypoxic/ischemic hypotensive cerebrovasodilator impairment (76). Results of that study (76) show that coadministration of NOC/oFQ, in a concentration observed in cortical periarachnoid CSF after H/I, 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. Hypotensive pial artery dilation was blunted by H/I but such dilation was partially protected by pretreatment with the putative NOC/oFQ receptor antagonist, [F/G] NOC/oFQ (1–13) $NH_2$ (76). Co-administration of the NMDA antagonist MK801 with NOC/oFQ partially prevented hypotensive pial dilation impairment. Similarly, pretreatment with MK801 partially protected hypoxic ischemia impairment of hypotensive pial dilation (76). 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 (76).

A second series of experiments was designed to determine the role of PKC activation in NOC/oFQ associated impairment of hypotension-induced pial artery dilation (77). Results of these studies show that the PKC inhibitor, chelerythrine, when coadministered with NOC/oFQ, did not di-

minish hypotensive pial dilation as greatly as compared to its absence (77). These data extend previous observations (70) to indicate that NOC/oFQ can inhibit the vascular response to several dilator stimuli via PKC activation. Additional data show that the chelerythrine partially prevented diminished hypotensive pial dilation post H/I (77). These data indicate the involvement of PKC activation in H/I impairment of hypotensive pial artery dilation.

Additional experiments were designed to characterize the role of $O^-_2$ generation in NOC/oFQ associated impairment of hypotensive pial artery dilation in sham control animals as well as the role for $O_2^-$ in H/I impairment of hypotensive dilation. Results of these studies show that the $O_2^-$ scavenger polyethylene glycol superoxide dismutase and catalase (SODCAT) partially protected decremented hypotensive pial artery dilation both when coadministered with NOC/oFQ in sham control animals as well as after H/I (77). These results indicate that $O_2^-$ formation contributes to NOC/oFQ-induced impairment of hypotensive pial dilation in sham control animals. Generation of $O_2^-$ also appears to contribute to H/I-associated impairment of hypotensive pial dilation. Because on a percentage basis SODCAT prevented hypotensive pial dilation impairment to a significantly greater extent than chelerythrine post H/I, these data suggest that $O_2^-$ generation is downstream of PKC activation (77).

Although uncertain as to the mechanism that might link CSF NOC/oFQ release post-H/I, PKC activation, and $O_2^-$ generation to impaired hypotensive pial artery dilation during reperfusion postinsult, one possibility could relate to the previously observed ability of NOC/oFQ to generate $O_2^-$ via PKC activation and thereby contribute to H/I impairment of NMDA induced pial artery vasodilation (70). Therefore, NOC/oFQ may active PKC to generate $O_2^-$, which impairs NMDA pial dilation to contribute to hypotensive dilator impairment post H/I. In that case, NOC/oFQ induced impairment of NMDA dilation might physiologically antagonize pial artery dilation during hypotension. Alternatively, other as yet, undefined, nonvascular associated events such as modulated signal transduction cascades may account for the concomitant roles for NOC/oFQ, NMDA, PKC, and $O_2^-$ in impaired hypotensive pial artery dilation following H/I.

PKC is an intracellular enzyme known to play an important role in signal transduction from the outside to the inside of cells (78). Activation of PKC elicits vasoconstriction and is thought to contribute to the development of vasospasm after subarachnoid hemorrhage (79). PKC activation has also been observed to occur after cerebral ischemia (80), whereas such activation can additionally result in the release of $O_2^-$ (70). Although previous studies have noted an association between $O_2^-$ generation and impaired hypotensive cerebrovasodilation following traumatic brain injury (81), results of this study (77) extend previous observation to indicate that the release of the opioid NOC/oFQ after H/I could link $O_2^-$ generation and PKC activation to impaired hypotensive pial dilation post insult.

**Traumatic Brain Injury in the Newborn.** *General Considerations.* Traumatic brain injury is one of the major causes of morbidity, mortality, and pediatric intensive care unit admissions of children today (82). Although the effects of traumatic brain injury have been well described for adult animal models (83, 84), few have investigated these effects in the newborn or have characterized such effects as a function of age using a single model of injury. For example, Adelson *et al.* (85) described the motor and cognitive functional deficits following diffuse traumatic brain injury using a weight drop model in the immature rat. Similarly, Smith *et al.* (86) described the role of $O_2^-$ in brain injury using a new 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 as a model of traumatic injury (84). More recently, Prins *et al.* (87) characterized the effects of FPI on several parameters, including mortality, intracranial pressure, and mean arterial blood pressure in the developing and adult rat. Other earlier studies had compared the cerebral hemodynamic effects of FPI in newborn (1–5 days old) and juvenile (3–4 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 (88). Moreover, systemic arterial blood pressure increases in the juvenile pig after brain injury, consistent with other adult studies (83), whereas it decreases in the newborn pig (88). 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 hr). Equally important, the above studies did not investigate the mechanisms for such age-related differences post-FPI. Finally, although FPI produces cerebral hypoperfusion and not ischemia, it still is considered to be a good mimic of shaken impact syndrome, an example of child abuse.

Several recent studies have been designed to characterize the contribution of NOC/oFQ to the cerebral hemodynamic effects of FPI. For example, results of one study show that cortical periarachnoid CSF NOC/oFQ concentration was elevated within 1 hr, remained elevated for 72 hr, but returned to control value within 168 hr post-FPI in the newborn pig (89). Reductions in pial artery diameter after FPI were evident for at least 8 hr in acute studied animals, whereas reductions in cerebral blood flow in all eight regions investigated were evident for at least 72 hr; blood flow returned to control value within 168 hr in the newborn (89). Interestingly, topical NOC/oFQ-induced pial dilation was reversed to vasoconstriction and such dilation was not fully returned to control until 168 hr postinsult. Systemic administration of the putative NOC/oFQ receptor antagonist [F/G] NOC/oFQ (1–13) $NH_2$ before FPI partially restored the decremented blood flow and pial artery diameter. Taken

together, these data suggest that impaired NOC/oFQ mediated pial dilation and elevated CSF NOC/oFQ concentration both contribute to altered hemodynamics after FPI (89).

In contrast, several differences in the observed parameters described above were noted when comparing these results in the juvenile versus the newborn pig (89). First, the CSF concentration for NOC/oFQ was an order of magnitude less in the juvenile versus the newborn and such elevated levels were present for a shorter period of time post-FPI (89). Second, NOC/oFQ-mediated dilation was reversed to vasoconstriction in the newborn post-FPI whereas such dilation was only attenuated in the juvenile (89). Third, as observed previously (88, 90), pial artery diameter and cerebral blood flow were reduced to a greater extent and for a longer period of time after FPI in the newborn versus the juvenile. Such differences could relate to the greater release and role of NOC/oFQ in altered cerebral hemodynamics in the newborn (89). Mechanisms involved in NOC/oFQ contribution to impaired control hemodynamics after brain injury are currently uncertain. However, although it is more understandable as to how reversal of NOC/oFQ from a vasodilator to a vasoconstrictor could contribute to a reduced cerebral blood flow after FPI in the newborn, it is less obvious and really uncertain as to how a diminished dilation to NOC/oFQ after FPI results in reduced cerebral blood flow in the juvenile.

On the basis of interspecies extrapolation of brain growth curves (91), the age period of newborn pigs chosen in the present study may approximate the newborn to infant time period in the human. Correspondingly, the age period for the juvenile pig chosen in the present study may correlate to that of a human child 5–8 years of age (91). 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 well depend on differences in the composition and compliance of the newborn and juvenile brain. Additionally, it is unclear how developmental parameters such as brain water content, skull dimensions, or suture elasticity will affect the biomechanics of the fluid wave pulse delivered to the brains of these two age groups.

Previous studies have characterized the hemodynamic effects of brain injury in adult animals. Although somewhat variable, most have observed reductions in cerebral blood flow and pial vessel diameter. For instance, decreased cerebral blood flow was observed in the adult monkey, cat, and rat (84, 92, 93), but elevated cerebral blood flow has been observed briefly in the adult cat (93). Such differences in cerebral hemodynamic response to brain injury could be caused by choice of anesthetic, species differences, or, more likely, a relatively different position on the stimulus response curve. For example, Unterburg *et al.* (94) observed that higher levels of brain injury in the adult cat were only associated with a reduction and not an increase in cerebral blood flow. Although fewer in number, previous studies have also characterized the effects of traumatic brain injury in immature animals. For example, Prins *et al.* (87) demonstrated that immature rats exhibited better functional outcome than adult rats after FPI. Similarly, Grundl *et al.* (95) reported that hypoperfusion was less severe after focal injury in immature than in adults rats.

*Mechanisms for Brain Injury Associated Impairment of NOC/oFQ Cerebrovasodilation.* Similar to that described above for H/I impairment of NOC/oFQ cerebrovasodilation, such impairment post FPI could also relate to an altered stimulated release of prostaglandins by this opioid. For example, NOC/oFQ stimulated release of 6-Keto-$PGF_1\alpha$ was blocked whereas NOC/oFQ stimulated release of $TXB_2$ was enhanced after FPI (96), similar to that observed after H/I (55). NOC/oFQ-induced pial artery dilation was reversed to vasoconstriction after FPI. However, pretreatment with the cyclooxygenase inhibitor indomethacin or the $PGH_2/TXA_2$ receptor antagonist SQ 29,548 reversed that NOC/oFQ-mediated vasoconstriction back to vasodilation; such dilator responses were partially returned to control for indomethacin and fully returned to control for SQ29,548-pretreated animals (96). Therefore, the biochemical data support and corroborate the pharmacologic data and indicate that impaired NOC/oFQ dilation results post-FPI, at least in part, from blunted stimulus-induced release of $PGI_2$ and accentuated release of $TXA_2$ (96).

*Role of NOC/oFQ in Brain Injury-Associated Impairment of NMDA and Opioid Cerebrovasodilation.* Glutamatergic system hyperactivity has been demonstrated in animal models of traumatic brain injury, whereas NMDA receptor antagonists have been shown to protect against experimental brain injury (97, 98). However, although cerebral hemodynamics postinsult is thought to correlate with neurologic status, little attention has been given to the role of NMDA vascular activity in the sequelae of traumatic brain injury. Although the precise concentration at the receptor level is uncertain, co-administration of NOC/oFQ ($10^{-10}$M), the CSF concentration after FPI in the juvenile pig, with NMDA or glutamate diminished the pial artery dilation induced by these excitatory amino acids (99). Co-administration of NOC/oFQ ($10^{-9}$M), the CSF concentration after FPI in the newborn pig, further diminished NMDA and glutamate pial artery dilation (99). These data suggest that such concentrations of this opioid observed following FPI in the newborn could have physiologic 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 hr postinsult in the newborn. Such reversal was maintained for at least 8 hr with mild vasodilation reemerging at 72 hr and vasodilation being fully restored at 168 hr 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) $NH_2$

partially restored decremented NMDA and glutamate pial dilation observed post insult. Taken together, these data suggest that NOC/oFQ contributes to impaired NMDA and glutamate mediated pial artery dilation after FPI (99).

In contrast, there were several differences in the observed parameters described above after FPI in the juvenile versus that after this insult in the newborn. For example, NMDA- and glutamate-mediated vasodilation was not reversed to vasoconstriction but only attenuated at 1 hr post-FPI in the juvenile (99). Such responses were fully restored within 8 hr postinsult in the juvenile compared to 168 hr 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., $10^{-10}$ M) elicited similar inhibition of excitatory amino acid induced pial artery dilation in the newborn and juvenile. These data suggest that whereas 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 that in juveniles (99).

Mechanisms by which NOC/oFQ might alter excitatory amino acid induced pial artery dilation as observed in the above study are currently uncertain. A working model, however, might involve cyclooxygenase dependent oxygen free radical generation by NOC/oFQ (100). Such free radical generation occurs after FPI and contributes to post insult altered cerebrovascular control (100, 101).

Alternatively, other neurohormones released after traumatic brain injury might contribute to impaired excitatory amino acid vasodilation by themselves or via modulatory interactions with NOC/oFQ. For example, NOC/oFQ and vasopressin appear to interact, though the nature of such interaction is less well understood (10). Interestingly, CSF vasopressin concentration is elevated following FPI (2) and contributes to the release of NOC/oFQ following such an insult (102). The greater release of vasopressin following FPI in the newborn contributes to the corresponding greater release of NOC/oFQ in the newborn versus the juvenile (102). Moreover, vasopressin also contributes to the impairment of NMDA cerebrovasodilation after brain injury to a greater extent in the newborn versus juveniles (102). Although the mechanism for such vasopressinergic modulation of NMDA and glutamate vasodilation after FPI is uncertain, one possibility could relate to a change in the vascular response to vasopressin following this insult, resulting in physiologic antagonism of excitatory amino acid vasodilation. Specifically, vasopressin reverses from a dilator to a constrictor after FPI (2). Such vasoconstriction could therefore oppose the ability of NMDA and glutamate to vasodilate. Alternatively, vasopressin could also indirectly alter NMDA and glutamate vasodilation via its ability to release CSF NOC/oFQ (102) which, in turn, generates $O_2^-$ (100). The latter mechanism, then, might also contribute to the previously observed age dependent impairment of NMDA vasodilation after FPI by NOC/oFQ (102).

Endothelin-1 (ET-1), a peptide with potent vasoconstrictor properties (103), elicits pial artery constriction in the piglet (104). CSF ET-1 concentration is elevated after FPI and contributes to cerebrovascular disregulation in the piglet following such an insult in an age dependent manner (90). Although ET-1 has been observed to modulate the actions of opioids such as dynorphin after FPI (105), the relationship between ET-1 and NOC/oFQ is largely uncertain. Interestingly, similar to its modulation by vasopressin described above, results of a recent study (106) show that ET-1, in concentrations present in CSF following FPI, contributes to the release of CSF NOC/oFQ following such an insult. The greater release of such ET-1 following FPI in the newborn (90) contributes to the corresponding greater release of NOC/oFQ in the newborn versus the juvenile (106). Moreover, ET-1 also contributes to the impairment of NMDA cerebrovasodilation after brain injury to a greater extent in newborns versus juveniles. These data suggest that ET-1 contributes to NOC/oFQ induced impairment of NMDA cerebrovasodilation after brain injury in an age dependent manner (106).

Although the mechanism for such ET-1 modulation of NMDA and glutamate vasodilation after FPI is uncertain, one possibility could relate to a change in the vascular response to ET-1 after this insult, resulting in physiologic antagonism of excitatory amino acid vasodilation. Specifically, ET-1 induced pial artery dilation at low ET-1 concentrations is blocked whereas ET-1 induced vasoconstriction at higher agonist concentrations is potentiated after FPI in the piglet (107). Such vasoconstriction could therefore oppose the ability of NMDA and glutamate to vasodilate. Alternatively, ET-1 could also indirectly alter NMDA and glutamate vasodilation via its ability to release CSF NOC/oFQ (106). Therefore, it is possible that ET-1 might indirectly impair NMDA and glutamate vasodilation via its ability to enhance NOC/oFQ release, which in turn, generates $O_2^-$ after FPI.

However, ET-1 has also been observed to impair cerebral hemodynamic control following FPI via activation of PKC (105). In unrelated studies, PKC activation has been observed to generate $O_2^-$ and to contribute to such generation after FPI (101). Therefore, it is also possible that ET-1 may impair NMDA dilation via $O_2^-$ generation independent of NOC/oFQ release. Figure 1 summarizes the above potential mechanisms for impairment of NMDA pial artery dilation following FPI.

The CSF concentration of other opioids has been observed to be elevated following FPI (2). Interestingly, recent

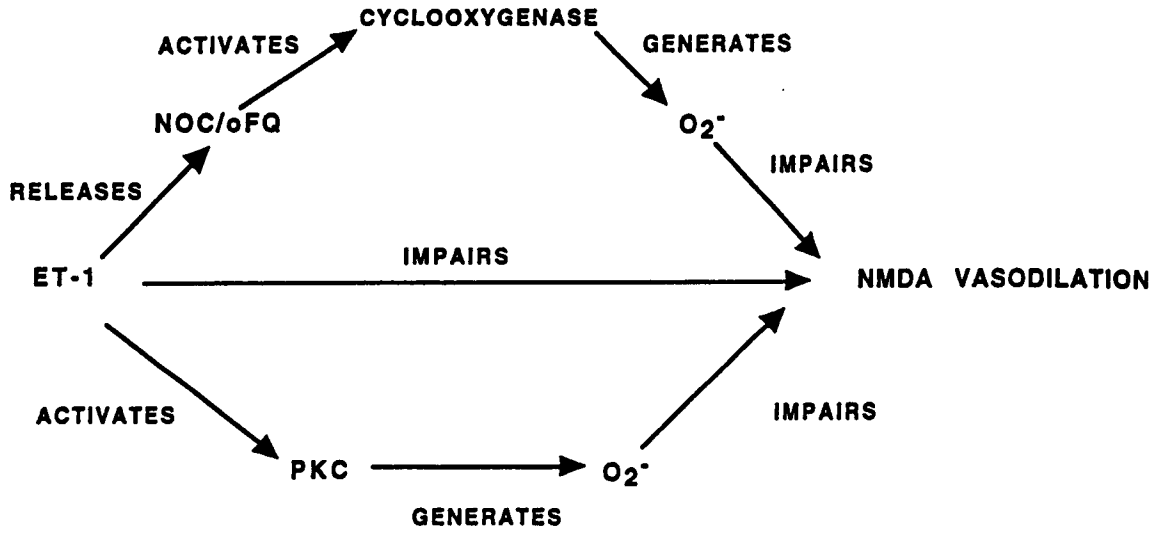


**Figure 1** Hypothetical mechanism for ET-1 contribution to NOC/oFQ induced impairment of NMDA cerebrovasodilation

studies have indicated that NOC/oFQ may interact, either positively or negatively, with the release of other opioids such as methionine enkephalin (108). However, the vascular interaction between NOC/oFQ and opioids such as methionine enkephalin is less well understood. Results of a final study show that co-administration of NOC/oFQ, in a concentration observed in CSF following FPI ($10^{-10}$ M), with either methionine enkephalin, leucine enkephalin, or dynorphin diminished the pial artery dilation induced by these opioids (109). A second series of experiments, then, were designed to determine the functional significance of the above noted interaction of NOC/oFQ with other opioids. The results of these studies showed that FPI attenuated methionine enkephalin and leucine enkephalin induced pial artery vasodilation while dynorphin was reversed from a dilator to a vasoconstrictor, consistent with other previously published studies (2). Such impaired opioid mediated vasodilation occurred concomitant with the elevation of CSF NOC/oFQ concentration. In contrast, the putative NOC/oFQ receptor antagonist [F/G] NOC/oFQ (1–13) $NH_2$ partially restored decremented methionine enkephalin and leucine enkephalin pial artery dilation observed post insult (109). Additionally [F/G] NOC/oFQ (1–13) $NH_2$ also restored dynorphin back to being a vasodilator post FPI. Taken together, these data suggest that NOC/oFQ contributes to impaired opioid mediated pial artery dilation following FPI (109).

## Concluding Remarks

NOC/oFQ appears to contribute to the regulation of the cerebral circulation. Its influence, however, is subtle during physiologic conditions but more robust during pathophysiologic conditions. For example, NOC/oFQ contributes to reductions of cerebral blood flow observed following hypoxia/ischemia or traumatic brain injury. In the latter pathologic state, the role of NOC/oFQ in injury related reductions in cerebral blood flow is age dependent, with a correspondingly greater contribution in the newborn versus the juvenile. In both pathologic conditions, however, NOC/oFQ interacts with other vasoactive systems/signal transduction pathways in a complex manner to result in impaired cerebral hemodynamics. Finally, because much of these data were accumulated in only one species, caution is urged regarding extrapolation to the human condition.


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