Relationship between nociceptin/orphanin FQ and cerebral hemodynamics after hypoxia-ischemia in piglets

WILLIAM M. ARMSTEAD
Departments of Anesthesia and Pharmacology, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Armstead, William M. Relationship between nociceptin/orphanin FQ and cerebral hemodynamics after hypoxia-ischemia in piglets. Am. J. Physiol. Heart Circ. Physiol. 278: H477–H483, 2000.—This study was designed to characterize the role of the newly described endogenous opioid nociceptin/orphanin FQ (NOC/oFQ) in reduced cerebral blood flow (CBF) observed after ischemia-reperfusion (I/R) and combined hypoxia and ischemia-reperfusion (H-I/R), as a function of time after onset of reperfusion in newborn pigs equipped with a closed cranial window. Global cerebral ischemia (20 min) was induced via elevation of intracranial pressure, whereas hypoxia (10 min) decreased PO₂ to 35 ± 3 mmHg with unchanged PCO₂. I/R elevated cerebrospinal fluid (CSF) NOC/oFQ from 67 ± 4 to 266 ± 29 pg/ml within 1 h, whereas values returned to control level within 4 h of reperfusion. H-I/R elevated CSF NOC/oFQ to 483 ± 67 pg/ml within 1 h, and such values returned slowly to control level within 12 h of reperfusion. Topical NOC/oFQ (10⁻⁸ M, 10⁻⁶ M)-induced vasodilation was attenuated by I/R and reversed to vasoconstriction by H-I/R at 1 h of reperfusion (control, 9 ± 1 and 16 ± 1%; I/R, 3 ± 1 and 6 ± 1%; H-I/R, −6 ± 1 and −11 ± 1%). Such altered dilation returned to control values within 4 h in I/R animals and within 12 h in H-I/R animals. Blood flow in the cerebrum was reduced from 58 ± 4 to 33 ± 2 ml·min⁻¹·100 g⁻¹ within 1 h and returned to control values within 4 h in I/R animals. In animals pretreated with [F/G]NOC/oFQ(1–13)-NH₂, an NOC/oFQ antagonist, however, CBF only fell to 43 ± 3 ml·min⁻¹·100 g⁻¹ at 1 h of reperfusion. Similar observations were made in H-I/R animals. These data suggest that an elevated CSF NOC/oFQ concentration and altered vascular responsiveness to this opioid contribute to reductions in CBF observed after either I/R or H-I/R.

newborn; cerebral circulation; opioids

EPISTODES OF INADEQUATE OXYGEN SUPPLY to the brain can result in significant neurological sequelae. Babies are frequently exposed to hypoxic-ischemic insults during the perinatal period. One contributor to neurological 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 (16–18). Less, however, is known about the cerebrovascular consequences of combined hypoxia and ischemia or about potential mechanisms for such altered cerebral hemodynamics.

Opioids have been observed to be important in the control of the cerebral circulation of the piglet during physiological and pathological conditions (3). During the last five years, several groups have isolated and cloned a new G protein-coupled receptor that showed high homology with opioid receptors (7, 10, 21). This opioid-like receptor, however, displayed no affinity for opioid ligands and remained an “orphan” until late 1995. At that time, two independent groups (20, 22) identified a 17-amino acid peptide that did not bind to the classic opioid receptors (µ, δ, and κ) but that activated the orphan receptor in a nanomolar concentration range and would therefore be considered the endogenous ligand for the orphan receptor (14). This peptide was named orphanin FQ by Reinscheid et al. (22) because its sequence begins with phenylalanine (F) and ends with glutamine (Q). The same peptide was called nociceptin by Meunier et al. (20) 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 ORL-1 (for opioid receptor-like 1) and its endogenous ligand, NOC/oFQ (for nociceptin/orphanin FQ). 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 (5). 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 (19). Additionally, NOC/oFQ has been observed to elicit vasodilation in the systemic and hindquarter vascular beds of the adult rat (6, 8, 9, 11, 13). Recently, NOC/oFQ has been observed to elicit pial artery vasodilation in the newborn pig (4). However, nothing is known about the role of NOC/oFQ in the physiological or pathophysiological control of cerebral hemodynamics. Although somewhat controversial (12, 15), the identification of an NOC/oFQ-receptor antagonist, [F/G]NOC/oFQ(1–13)-NH₂, and its demonstrated selectivity for NOC/oFQ in the piglet cerebral circulation (4) have resulted in the development of an avenue for the characterization of the functional significance of this newly described opioid.

Therefore, this study was designed to characterize the role of NOC/oFQ in the reduced cerebral blood flow observed after ischemia-reperfusion (I/R) and combined hypoxia and I/R as a function of time after the...
onset of reperfusion in the newborn pig. Thus, it is hypothesized that the vasodilator response to NOC/oFQ is either reduced or reversed to vasoconstriction by I/R to contribute to the reduced cerebral blood flow that follows this insult.

METHODS

Newborn (1–5 days 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. Animals were sedated with ketamine hydrochloride (33 mg/kg) and acepromazine (3.3 mg) intramuscularly. Anesthesia was maintained with α-chloralose (30–50 mg/kg, supplemented with 5 mg·kg⁻¹·h⁻¹ iv). Catheters were inserted into two femoral arteries to monitor blood pressure, sample for blood gas tensions and pH, and serve as a reference withdrawal for microsphere measurements of cerebral blood flow. Drugs to maintain anesthesia were administered through a third catheter placed in a femoral vein. A fourth catheter was placed in the left ventricle via the carotid artery for microsphere injection. 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, monitored with a rectal ultrasound axillary temperature probe. Vascular diameter was measured with a video microscope. Vascular diameter was measured with a video microscope, a television camera mounted on the microscope, and a video output screen. Vascular diameter was measured with a video microscler. For production of cerebral ischemia, a hollow stainless steel bolt was implanted in a small (2 mm) hole in the skull.

Blood flow within the cerebrum was measured using radioactively labeled microspheres. These methods have been used in this laboratory to measure blood flow in both anesthetized and conscious animals under a variety of experimental conditions. Briefly, a known amount of radioactivity in 15-µm microspheres (300,000–800,000 spheres) was injected into the left ventricle, and the injection line was flushed with 3 ml of saline. Withdrawal of reference blood samples was begun 15 s before microsphere injection and continued for 2 min after the injection. The reference withdrawal rate was 1.03 ml/min. After each experiment, the pig was killed and the brain removed and weighed. The brain was subdivided into major regions, and samples were counted in a gamma counter. The energy from each nuclide was separated by differential spectrophotometry. Aliquots of the whole microsphere solutions injected were used for overlap calculations. The count in each milliliter per minute of blood flow was determined by dividing the counts in the reference withdrawal by the rate of reference withdrawal. Thus blood flow to any organ at the time of microsphere injection can be calculated as $Q = C \times R \times CR^{-1}$, where Q is organ blood flow (in ml/min), C is the counts per minute (cpm) in the tissue sample, R is the rate of withdrawal of the reference blood sample (in ml/min), and CR is the total counts in the reference arterial blood sample.

Protocol. Two types of pial arterial vessels, small arteries (resting diameter 120–160 µm) and arterioles (resting diameter 50–70 µm), were examined in each animal to determine whether segmental differences in the effects of hypoxia-ischemia could be identified. Pial arterial vessels were observed with a dissecting microscope, a television camera, and a video monitor. Blood flow in pial arterioles, viewed with a dissecting microscope and a video monitor, was stopped completely on elevation of intracranial pressure and did not resume until the pressure was lowered. 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 mmHg. As the cerebral ischemic response subsided, the shed blood was returned to the animal. Cerebral ischemia was maintained for 20 min. In animals exposed to combined hypoxia and I/R, hypoxia (PO₂, 3–5 mmHg) was produced for 10 min, which was followed by the total ischemia protocol as described above.

Six types of experiments were performed: 1) sham control (bolts inserted but intracranial pressure not increased, n = 6), 2) I/R (n = 6), 3) hypoxia and I/R (H-I/R, n = 6), 4) sham control pretreated with the NOC/oFQ-receptor antagonist [F/G]NOC/oFQ(1–13)-NH₂ (1 mg/kg iv and 10⁻⁶ M topical, n = 6), 5) I/R with NOC/oFQ antagonist pretreatment (n = 6), and 6) H-I/R with NOC/oFQ antagonist pretreatment (n = 6). Topical NOC/oFQ (10⁻⁸ M, 10⁻⁶ M) (Phoenix Pharmaceuticals) was administered before intervention (time 0) and at 1 and 4 h of reperfusion in I/R animals or at 1, 4, 8, and 12 h of reperfusion in H-I/R animals. Responses at the same intervals were obtained in sham control animals. Because baseline pial artery diameter changed as a result of the I/R or H-I/R intervention, data were calculated as the percent change from baseline to normalize such differences. The NOC/oFQ antagonist was administered 20 min before ischemia. The vehicle used in this laboratory to measure blood flow in both anesthetized and conscious animals under a variety of experimental conditions. Briefly, a known amount of radioactivity in 15-µm microspheres (300,000–800,000 spheres) was injected into the left ventricle, and the injection line was flushed with 3 ml of saline. Withdrawal of reference blood samples was begun 15 s before microsphere injection and continued for 2 min after the injection. The reference withdrawal rate was 1.03 ml/min. After each experiment, the pig was killed and the brain removed and weighed. The brain was subdivided into major regions, and samples were counted in a gamma counter. The energy from each nuclide was separated by differential spectrophotometry. Aliquots of the whole microsphere solutions injected were used for overlap calculations. The count in each milliliter per minute of blood flow was determined by dividing the counts in the reference withdrawal by the rate of reference withdrawal. Thus blood flow to any organ at the time of microsphere injection can be calculated as $Q = C \times R \times CR^{-1}$, where Q is organ blood flow (in ml/min), C is the counts per minute (cpm) in the tissue sample, R is the rate of withdrawal of the reference blood sample (in ml/min), and CR is the total counts in the reference arterial blood sample.
and $B_n = (\text{average cpm of total binding tube} - \text{average cpm of nonspecific binding tube}).$

Statistical analysis. Pial arteriolar diameter, systemic arterial pressure, and NOC/oFQ levels were analyzed using ANOVA for repeated measures or t-test where appropriate. If the value was significant, the data were then analyzed by Fishers protected least significant difference test. An alpha 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 I/R and H-I/R on CSF NOC/oFQ concentration and pial artery reactivity. Experiments were initially designed to characterize the influence of I/R and H-I/R on CSF NOC/oFQ concentration. Cortical periarachnoid CSF NOC/oFQ was elevated within 1 h but returned to control value within 4 h of reperfusion in I/R animals (Fig. 1A). In H-I/R animals, initial hypoxia modestly elevated CSF NOC/oFQ (Fig. 1B). Subsequent I/R after initial hypoxia further elevated CSF NOC/oFQ (Fig. 1B). CSF NOC/oFQ was maximal within 1 h, began to drop within 4 h, and was at control level within 12 h of reperfusion (Fig. 1B).

Topical NOC/oFQ ($10^{-6}, 10^{-5} \text{M}$) elicited reproducible pial small artery (120–160 µm) and arteriole (50–70 µm) dilation over a 12-h period in sham control animals (data not shown). NOC/oFQ-induced pial dilation was diminished within 1 h but returned to control value within 4 h of reperfusion in I/R animals (Fig. 2). Pretreatment with [F/G]NOC/oFQ(1–13)-NH$_2$ (1 mg/kg iv and $10^{-6}$ M topical) blocked NOC/oFQ-induced dilation before (control) and after I/R (Fig. 3). Systemic administration of this antagonist alone also blocked NOC/oFQ-mediated dilation. This NOC/oFQ antagonist had no effect on pial artery diameter by itself (141 ± 6 vs. 140 ± 5 µm, $n = 6$).

In contrast, NOC/oFQ-induced dilation was reversed to pial artery vasoconstriction at both 1 and 4 h of reperfusion after H-I/R (Fig. 4). At 8 h of reperfusion such vasoconstriction had returned to modest vasodilation, whereas at 12 h of reperfusion NOC/oFQ dilation was not different from that observed before the insult (Fig. 4). The NOC/oFQ antagonist [F/G]NOC/oFQ(1–13)-NH$_2$ (1 mg/kg iv and $10^{-6}$ M topical) blocked NOC/oFQ vascular activity for ≤12 h of reperfusion after H-I/R (data not shown). Hypoxia by itself dilated pial small arteries from 136 ± 8 to 165 ± 9 µm ($n = 6$).

Role of NOC/oFQ in cerebral blood flow reductions after I/R and H-I/R. Blood flow in the cerebral was decreased within 1 h, but such values returned to control level within 4 h of reperfusion in I/R animals (Fig. 5). [F/G]NOC/oFQ(1–13)-NH$_2$ had no effect on blood flow in the cerebral before or at 4 h of reperfusion in I/R animals (Fig. 5). However, [F/G]NOC/oFQ(1–13)-NH$_2$ partially restored the decremented blood flow in the cerebral observed at 1 h of reperfusion (Fig. 5).

In contrast, blood flow in the cerebral was decreased within 1 h, and such reduction was maintained for at least 8 h of reperfusion in H-I/R animals (Fig. 6). Reductions in blood flow at 1 h of reperfusion were significantly greater in H-I/R than in I/R animals (62 ± 2 vs. 42 ± 3%). Similar to its action in I/R animals, however, [F/G]NOC/oFQ(1–13)-NH$_2$ partially restored the decremented blood flow in H-I/R animals (Fig. 6).

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 PO$_2$ to 35 ± 3 mmHg, whereas the pH, PCO$_2$, and mean arterial blood pressure values were unchanged. Values for pH, PCO$_2$, PO$_2$, and mean arterial blood pressure were 7.46 ± 0.02, 36 ± 3 mmHg, 94 ± 5 mmHg, and 70 ± 5 mmHg at the start of experiments versus 7.45 ± 0.02, 37 ± 4 mmHg, 93 ± 5 mmHg, and 68 ± 5 mmHg, respectively, at the end of experiments. Systemic infusion of [F/G]NOC/oFQ(1–13)-NH$_2$ had no effect on mean arterial blood pressure or blood chemistry. Additionally, there were no group differences in either blood pressure or blood chemistry values.
Results of the present study show that cortical periarachnoid CSF NOC/oFQ concentration was elevated within 1 h but returned to control value within 4 h of reperfusion after I/R. Blood flow in the cerebrum was also decreased within 1 h of reperfusion but returned to control value within 4 h of reperfusion. Interestingly, topical NOC/oFQ-induced pial artery dilation was diminished within 1 h of reperfusion, but such dilation was not different from that observed before I/R within 4 h of reperfusion. Systemic administration of the putative NOC/oFQ-receptor antagonist [F/G]NOC/oFQ(1–13)-NH₂ before ischemia as well as at 1 and 4 h of reperfusion, these data indicate that systemically administered [F/G]NOC/oFQ(1–13)-NH₂ crosses the blood-brain barrier in sufficient quantity to inhibit responses to the agonist NOC/oFQ for at least 4 h. However, [F/G]NOC/oFQ(1–13)-NH₂ did not affect cerebral blood flow before or at 4 h of reperfusion after ischemia, suggesting that NOC/oFQ probably contrib-

DISCUSSION

Fig. 2. A: influence of NOC/oFQ (10⁻⁸ M, 10⁻⁶ M) on pial small artery diameter before (control) and at 1 and 4 h post-I/R. B: influence of NOC/oFQ on pial arteriole diameter before (control) and at 1 and 4 h post-I/R (n = 6). *P < 0.05 compared with control.

Fig. 3. A: influence of NOC/oFQ (10⁻⁸ M, 10⁻⁶ M) on pial small artery diameter in [F/G]NOC/oFQ(1–13)-NH₂-pretreated animals before (control) and at 1 and 4 h post-I/R. B: influence of NOC/oFQ on pial arteriole diameter in [F/G]NOC/oFQ(1–13)-NH₂-pretreated animals (n = 6). *P < 0.05 compared with absence of [F/G]NOC/oFQ(1–13)-NH₂ (see Fig. 2).
utes little to cerebral hemodynamics during resting physiological conditions. Given that pial small arteries exhibited about the same percent decrease in responsiveness to NOC/oFQ at 1 h of reperfusion after ischemia as that observed with pial arterioles, these data also suggest that there are probably minimal regional segmental vascular differences in altered NOC/oFQ activity after I/R.

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 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. Hypoxia by itself also modestly elevated CSF NOC/oFQ concentration. Additionally, blood flow was decreased in percentage to a greater extent at 1 h of reperfusion and remained depressed for a longer period of time (at least 8 h) 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 h of reperfusion after H-I/R. At 8 h of reperfusion such vasoconstriction was returned to modest vasodilation, whereas at 12 h of reperfusion NOC/oFQ dilation was no different from that observed before the insult. Systemically administered [F/G]NOC/oFQ(1–13)-NH2 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 elevate CSF NOC/oFQ concentration and alter 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 as to 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 as to how a diminished dilation to NOC/oFQ after I/R results in reduced cerebral blood flow.

Global cerebral ischemia in a piglet model has been previously observed to result in reductions in blood flow of the cerebrum and altered pial artery dilation to stimuli such as hemorrhagic hypotension and hypercapnia (16–18). However, such ischemic effects are not nonselective in that although responses to these stimuli
were impaired, others (e.g., isoproterenol) were not (16, 17). Hypoxia has also been observed to elevate the CSF concentration of other opioids such as methionine enkephalin, which contributes to, and dynorphin, which opposes, hypoxic pial artery dilation (1–3). The present study, however, was not designed to investigate the contribution of NOC/oFQ to hypoxic pial artery dilation. The results of this study are, though, the first to describe the contribution of NOC/oFQ to altered cerebral hemodynamics after I/R or H-I/R.

Although many actions of NOC/oFQ have been described (19), little has been published on the functional significance of such actions because of the lack of an appropriate antagonist. Recently, however, a promising candidate for such a role has been described, [F/G]NOC/oFQ(1–13)-NH$_2$ (12). However, [F/G]NOC/oFQ(1–13)-NH$_2$ has also recently been observed to function as an agonist at the NOC/oFQ receptor when administered by intracerebroventricular injection in the conscious rat (15). Fortunately, results of a recent study (4) support its selectivity in the piglet cerebral circulation. For example, topical [F/G]NOC/oFQ(1–13)-NH$_2$ (10$^{-6}$ M) had no effect on the pial vascular responses to the endogenous opioids methionine enkephalin, leucine, enkephalin, dynorphin, and β-endorphin (3, 4). Similarly, [F/G]NOC/oFQ(1–13)-NH$_2$ had no effect on the synthetic opioids [d-Ala$_2$]-N-Me-Phe$_4$,Gly-ol$_5$ enkephalin, [d-Pen$_2$,5]-enkephalin, deltorphin, and U-50488 H$, \mu$, δ$, \kappa$-, and $\kappa$-selective opioid receptor agonists in the piglet cerebral circulation (3, 4). Other results showed that NOC/oFQ-induced pial artery dilation was unchanged by β-funaltrexamine, 7-benzylidenenaltrexone, naltrindole, norbinaltorphimine, and naloxone, agents shown to be selective $\mu$, δ$, \kappa$, and nonselective opioid receptor antagonists, respectively, in the piglet cerebral circulation (3, 4). NOC/oFQ was blocked, though, by topical [F/G]NOC/oFQ(1–13)-NH$_2$ (4). These results were reconfirmed for combined topically and systemically administered [F/G]NOC/oFQ(1–13)-NH$_2$ in the present study. These data indicate that NOC/oFQ and [F/G]NOC/oFQ(1–13)-NH$_2$ are selective agonist and antagonist, respectively, for the recently described ORL-1 receptor in the pial artery vascular system.

However, the experimental design of the present study did not allow for the identification of the cellular site of origin for NOC/oFQ detected in cortical periaqueductal CSF. Potential cellular sources include neurons, glia, vascular smooth muscle, and endothelial cells.

Opioids are important contributors to the regulation of the newborn pig cerebral circulation during physiological and pathological conditions (3). Because the present study did not characterize responses to NOC/oFQ after I/R or H-I/R in the juvenile or adult, it is uncertain whether similar results could be expected in the adult.

In conclusion, results of the present study suggest that elevated CSF NOC/oFQ concentration and altered vascular responsiveness to this opioid contribute to reductions in cerebral blood flow observed after either I/R or combined hypoxia and I/R.

This research was supported by grants from the National Institutes of Health, the American Heart Association (AHA), and the University of Pennsylvania Research Foundation. W. M. Armstead is an Established Investigator of the AHA.

Address for reprint requests and other correspondence: W. M. Armstead, Dept. of Anesthesia, Univ. of Pennsylvania, 3400 Spruce St., Philadelphia, PA 19104 (E-mail: armstew@mail.med.upenn.edu).

Received 14 J une 1999; accepted in final form 9 September 1999.

REFERENCES

18. Leffler, C. W., D. W. Busija, R. Mirro, W. M. Armstead, and D. G. Beasley. Effects of ischemia on brain blood flow and oxygen...


