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Am J Physiol Heart Circ Physiol 284:101-107, 2003. doi:10.1152/ajpheart.00457.2002

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J. W. Kiessling, D. B. Cines, A. A.-R. Higazi and W. M. Armstead

Am J Physiol Heart Circ Physiol, March 1, 2009; 296 (3): H862-H867.

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Differential role of PTK and ERK MAPK in superoxide impairment of KATP and KCa channel cerebrovasodilation

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Am J Physiol Regulatory Integrative Comp Physiol, July 1, 2003; 285 (1): R149-R154.

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PTK, MAPK, and NOC/oFQ impair hypercapnic cerebrovasodilation after hypoxia/ischemia

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Submitted 29 May 2002; accepted in final form 28 August 2002

Jagolino, Amanda L., and William M. Armstead. PTK, MAPK, and NOC/oFQ impair hypercapnic cerebrovasodilation after hypoxia/ischemia. *Am J Physiol Heart Circ Physiol* 284: H101–H107, 2003; 10.1152/ajpheart.00457.2002.—This study characterized the contributions of protein tyrosine kinase (PTK) and mitogen-activated protein kinase (MAPK) in nociceptin/orphanin FQ (NOC/oFQ)-induced impairment of hypercapnic pial artery dilation (PAD) after hypoxia/ischemia (H/I) in piglets equipped with a closed cranial window. NOC/oFQ (10^{-10} M cerebrospinal fluid H/I concentration) impaired hypercapnic PAD ($21 \pm 2\%$ vs. $13 \pm 1\%$). Coadministration of either of the PTK inhibitors genistein or tyrphostin A23 or the MAPK inhibitors U-0126 or PD-98059 with NOC/oFQ (10^{-10} M) partially prevented the inhibition of hypercapnic PAD compared with that observed in their absence ($21 \pm 2\%$ vs. $17 \pm 1\%$ for genistein). After exposure to H/I, PAD in response to hypercapnia was impaired, but pretreatment with either genistein, tyrphostin A23, U-0126, or PD-98059 partially protected such impairment ($17 \pm 1\%$ vs. $4 \pm 1\%$ vs. $9 \pm 1\%$ for sham control, H/I, and H/I + genistein pretreatment, respectively). These data show that PTK and MAPK activation contribute to NOC/oFQ-induced impairment of hypercapnic PAD. These data suggest that activation of PTK and MAPK is also involved in the mechanism by which NOC/oFQ impairs hypercapnic PAD after H/I.

newborn; cerebral circulation; opioids; signal transduction; protein tyrosine kinase; mitogen-activated protein kinase; nociceptin/orphanin FQ

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 due to problems with delivery or respiratory management postdelivery (29). One contributor to neurological damage is thought to be cerebrovascular dysfunction.

Carbon dioxide is a powerful physiological regulator of the cerebral circulation (11). Previous studies have observed that hypercapnic pial artery dilation was blunted after global cerebral ischemia in the newborn pig (18). While impairment of prostaglandin-associated vascular responses is thought to contribute to such altered hypercapnic dilation postischemia (18, 19, 21, 22), the exact mechanism remains uncertain.

Prostaglandins are thought to be important in the regulation of the neonatal cerebral circulation (15). Prostaglandins are released during hypercapnia and have a permissive role in hypercapnic vasodilation (23). Protein tyrosine kinase (PTK) is an intracellular messenger involved in signal transduction and is released in response to injury (14). Interestingly, PTK is believed to modulate the permissive role of prostaglandins in hypercapnic conditions (27). Additionally, mitogen-activated protein kinase (MAPK) is a substrate for PTK and is thought to contribute to impaired cerebral hemodynamic control in pathological states such as ischemia (14). However, the role of PTK and MAPK activation in impaired hypercapnic dilation observed after cerebral hypoxia/ischemia is uncertain.

During the last 7 years, several groups have isolated and cloned a new G protein-coupled receptor that showed high homology with opioid receptors (7, 9, 26). The peptide ligand for this receptor does not bind to classical opioid receptors (μ , δ , κ) and was named orphanin FQ by Reinscheid et al. (28) because its sequence begins with phenylalanine (F) and ends with a glutamine (Q). The same peptide was called nociceptin by Meunier et al. (25) 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 nociceptin/orphanin FQ (NOC/oFQ) in the physiological or pathophysiological control of cerebral hemodynamics. Recent studies have shown that the cerebrospinal fluid (CSF) concentration of NOC/oFQ is elevated to $\sim 10^{-10}$ M after hypoxia/ischemia in the piglet (3). Interestingly, it has also been observed to contribute to the reduction in cerebral blood flow that occurs after hypoxia/ischemia (3). More recently, NOC/oFQ has been observed to contribute to impaired hypercapnic dilation after hypoxia/ischemia (13). The mechanism whereby NOC/oFQ might contribute to altered hypercapnic dilation postinsult is unknown. Interestingly, however, NOC/oFQ may activate PTK (10).

Therefore, this study was designed to characterize the contributions of PTK and MAPK activation in NOC/oFQ-induced impairment of hypercapnic pial artery dilation after cerebral hypoxia/ischemia.

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MATERIALS AND METHODS

Newborn (1–5 days old) 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 MAC). 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 (rectal).

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 MgCl₂, 1.5 CaCl₂, 132 NaCl, 6.6 urea, 3.7 dextrose, and 24.6 NaHCO₃. This artificial CSF was warmed to 37°C and had the following chemistry: pH 7.33, PCO₂ 46 mmHg, and PO₂ 43 mmHg, which was similar to that of endogenous CSF. Pial arterial vessels were observed with a dissecting microscope, a television camera mounted on the microscope, and a video output screen. Vascular diameter was measured with a video microscaler. 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 μ m) and arterioles (resting diameter 50–70 μ m), were examined to determine whether segmental differences in the effects of hypoxia/ischemia 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 artificial CSF containing a drug. Typically, 2–3 ml of CSF were flushed through the window over 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 (18–20). Briefly, total cerebral ischemia was accomplished by infusing artificial CSF into a hollow bolt in the cranium to maintain an intracranial pressure 15 mmHg greater than the numerical mean of systolic and diastolic arterial blood (20). 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 (20). 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. Hypoxia (PO₂ = 34 \pm 3 mmHg) was produced for 10 min before ischemia by decreasing the inspired O₂ via inhalation of N₂, which was immediately followed by the total ischemia protocol as described above after concomitantly restoring ventilation to room air.

Seventeen types of experiments were performed (all $n = 7$): 1) sham control; 2) NOC/oFQ pretreated; 3) NOC/oFQ +

genistein (10⁻⁶ M); 4) NOC/oFQ + genistein (10⁻⁵ M); 5) NOC/oFQ + tyrphostin A23 (10⁻⁶ M); 6) NOC/oFQ + U-0126 (10⁻⁶ M); 7) NOC/oFQ + U-0126 (10⁻⁵ M); 8) NOC/oFQ + PD-98059 (10⁻⁵ M); 9) NOC/oFQ + combined genistein (10⁻⁵ M) and U-0126 (10⁻⁵ M); 10) hypoxia/ischemia; 11) hypoxia/ischemia pretreated with genistein (10⁻⁶ M); 12) hypoxia/ischemia pretreated with genistein (10⁻⁵ M); 13) hypoxia/ischemia pretreated with tyrphostin A23; 14) hypoxia/ischemia pretreated with U-0126 (10⁻⁶ M); 15) hypoxia/ischemia pretreated with U-0126 (10⁻⁵ M); 16) hypoxia/ischemia pretreated with PD-98059; and 17) hypoxia pretreated with combined genistein (10⁻⁵ M) and U-0126 (10⁻⁵ M). Thus two structurally different PTK inhibitors (genistein and tyrphostin A23) and MAPK inhibitors (U-1026 and PD-98059) were used. In sham control animals, responses were obtained to hypercapnia initially and then again 60 min later. In NOC/oFQ-pretreated animals, responses were obtained to hypercapnia before NOC/oFQ was applied. After NOC/oFQ (10⁻¹⁰ M) was applied in these animals, responses to hypercapnia were obtained again 60 min later. Other control experiments involved obtaining responses to hypercapnia in animals pretreated with each of the inhibitors alone. Two levels of hypercapnia (low and high) were induced via inhalation of graded levels of a 10% CO₂-21% O₂-balance N₂ gas mixture to produce levels of PCO₂ of 50–60 mmHg for the low exposure and 70–80 mmHg for the high exposure. PTK and MAPK inhibitors were topically applied 30 min before either coadministration with NOC/oFQ or induction of hypoxia/ischemia.

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 test. An α -level of $P < 0.05$ was considered significant in all statistical tests. Values are presented as means \pm SE of absolute values or as percentages of change from control values.

RESULTS

Role of PTK and MAPK activation in NOC/oFQ-induced impairment of hypercapnic pial artery dilation. Two levels of hypercapnia (low and high) elicited reproducible graded pial small artery (120–160 μ m) and arteriole (50–70 μ m) dilation in sham control animals (data not shown). Pretreatment with NOC/oFQ (10⁻¹⁰ M) diminished pial dilation to both levels of hypercapnia (Fig. 1). On a percentage basis, NOC/oFQ inhibited low hypercapnic dilation similarly in pial small arteries and arterioles (35 \pm 5% vs 38 \pm 4%). However, the higher level of hypercapnia-induced dilation was inhibited modestly more in arterioles versus small arteries (50 \pm 4% vs. 35 \pm 6%). NOC/oFQ (10⁻¹⁰ M) by itself had no significant effect on pial small artery or arteriole diameter.

However, coadministration of either genistein (10⁻⁶ M) or tyrphostin A23 (10⁻⁵ M) with NOC/oFQ (10⁻¹⁰ M) partially prevented the inhibition of hypercapnic dilation compared with that observed in its absence (Fig. 1). The data from coadministration of tyrphostin A23 and NOC/oFQ, on a percentage basis, reflect a pial small artery and arteriole dilation impairment of 12 \pm 3% and 17 \pm 5% during the low hypercapnic challenge and 17 \pm 3% and 18 \pm 5% inhibition during the high hypercapnic challenge, respectively. These values are significantly different from those listed above in the

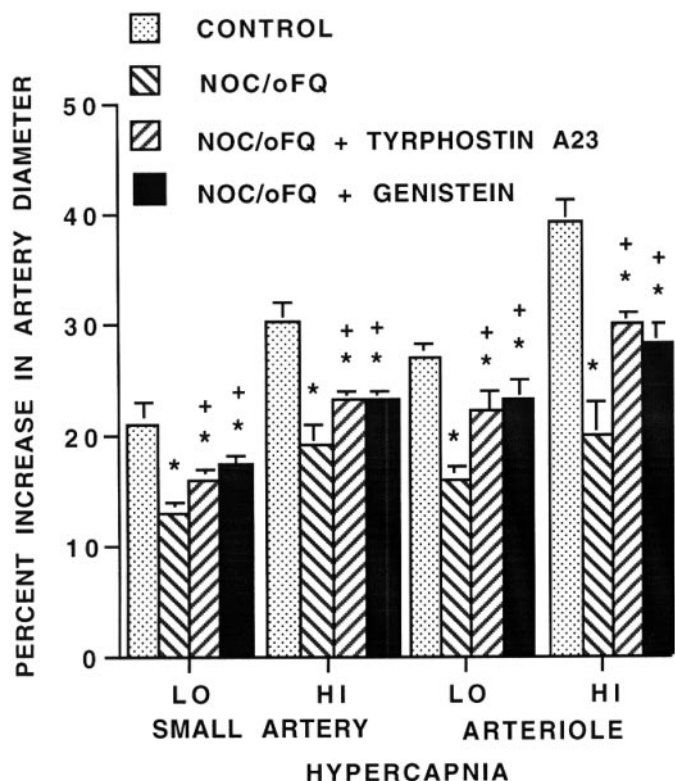


Fig. 1. Influence of low (lo) and high (hi) hypercapnia on pial small artery and arteriole diameter in the absence (control) and presence of nociceptin/orphanin FQ (NOC/oFQ; 10^{-10} M) after coadministration of NOC/oFQ and tyrphostin A23 (10^{-5} M) and after coadministration of NOC/oFQ and genistein (10^{-6} M); $n = 7$. * $P < 0.05$ compared with the corresponding control value; + $P < 0.05$ compared with the corresponding NOC/oFQ without genistein or tyrphostin A23 value.

absence of tyrphostin A23. Coadministration of another PTK inhibitor, genistein (10^{-6} M), with NOC/oFQ similarly partially restored decremented hypercapnic dilation compared with NOC/oFQ alone. These data reflect on a percentage basis a pial small artery and arteriole dilation impairment of $14 \pm 3\%$ and $15 \pm 3\%$ during the low hypercapnic challenge and $13 \pm 4\%$ and $13 \pm 4\%$ inhibition during the high hypercapnic challenge, respectively, during coadministration of genistein and NOC/oFQ. Administration of a higher concentration of genistein (10^{-5} M) with NOC/oFQ produced no further restoration of decremented hypercapnic dilation compared with that observed with genistein (10^{-6} M) (data not shown).

In addition, coadministration of either of the MAPK inhibitors U-0126 (10^{-6} M) or PD-98059 (10^{-5} M) with NOC/oFQ (10^{-10} M) also partially prevented the inhibition of hypercapnic dilation compared with that observed in its absence (Fig. 2). The data from coadministration of U-0126 and NOC/oFQ, on a percentage basis, reflect a pial small artery and arteriole dilation impairment of $17 \pm 6\%$ and $17 \pm 4\%$ during the low hypercapnic challenge and $20 \pm 4\%$ and $21 \pm 5\%$ inhibition during the high hypercapnic challenge, respectively. These values are significantly different from those listed above in the absence of U-0126. Ad-

ministration of a higher concentration of U-0126 (10^{-5} M) with NOC/oFQ produced no further restoration of decremented hypercapnic dilation compared with that observed with U-0126 (10^{-6} M) (data not shown). Similarly, combined administration of genistein (10^{-5} M) and U-0126 (10^{-5} M) did not elicit any further significant protection. Coadministration of PD-98059 with NOC/oFQ partially restored decremented hypercapnic dilation compared with NOC/oFQ alone as well. These data reflect on a percentage basis a pial small artery and arteriole dilation impairment of $23 \pm 6\%$ and $19 \pm 6\%$ during the low hypercapnic challenge and $14 \pm 6\%$ and $12 \pm 6\%$ inhibition during the high hypercapnic challenge, respectively, during coadministration of PD-98059 and NOC/oFQ. The values are also significantly different from the above listed in the absence of PD-98059.

Role of PTK and MAPK activation in impaired hypercapnia-induced pial artery dilation after hypoxia/ischemia. After exposure to hypoxia/ischemia, pial arterial dilation in response to hypercapnia was impaired (Fig. 3). On a percentage basis, these data reflect a pial small artery and arteriole dilation impairment of $80 \pm 3\%$ and $76 \pm 5\%$, respectively, during the low-level hypercapnia, and $77 \pm 5\%$ and $76 \pm 3\%$ impairment during the high-level hypercapnia. Pretreatment with either genistein (10^{-6} M) or tyrphostin A23 (10^{-5} M) before insult partially protected impairment of the

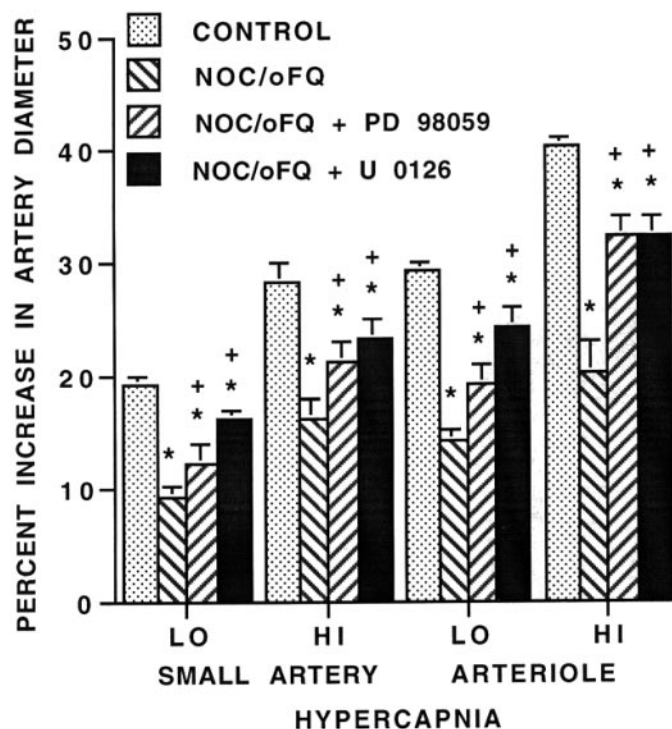


Fig. 2. Influence of low and high hypercapnia on pial small artery and arteriole diameter in the absence (control) and presence of NOC/oFQ (10^{-10} M) after coadministration of NOC/oFQ and PD-98059 (10^{-5} M) and after coadministration of NOC/oFQ and U-0126 (10^{-6} M); $n = 7$. * $P < 0.05$ compared with the corresponding control value; + $P < 0.05$ compared with the corresponding NOC/oFQ without PD-98059 or U-0126 value.

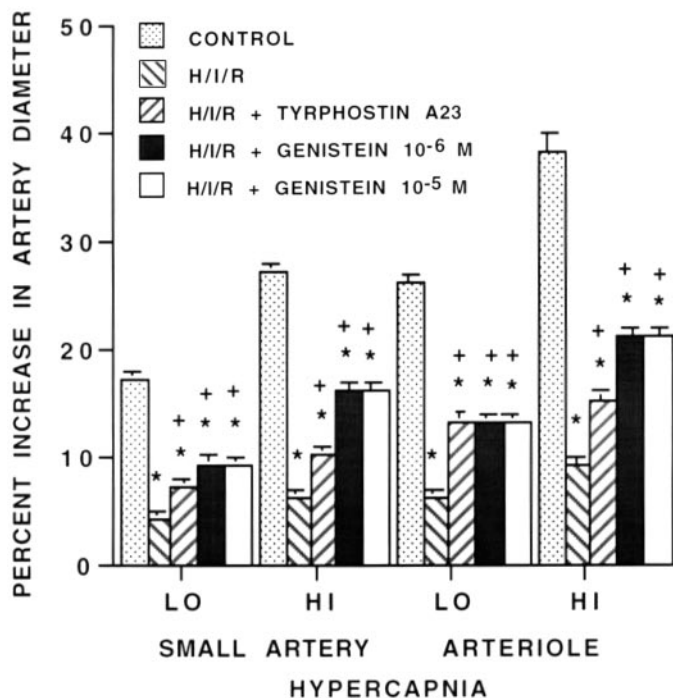


Fig. 3. Influence of low and high hypercapnia on pial small artery and arteriole diameter before (control), after hypoxia/ischemia-reperfusion (H/I/R), after hypoxia/ischemia in tyrphostin A23 (10^{-5} M)-pretreated animals, after hypoxia/ischemia in genistein (10^{-6} M)-pretreated animals, and after hypoxia/ischemia in genistein (10^{-5} M)-pretreated animals; $n = 7$. * $P < 0.05$ compared with the corresponding control value; + $P < 0.05$ compared with the corresponding hypoxia/ischemia nonpretreated value.

hypercapnic dilation after hypoxia/ischemia (Fig. 3). On a percentage basis, these data reflect a pial small artery and arteriole dilation impairment of $59 \pm 5\%$ and $51 \pm 4\%$ during low-level hypercapnia and $62 \pm 2\%$ and $62 \pm 3\%$ inhibition during high-level hypercapnia for tyrphostin A23-pretreated animals. These data also reflect a pial small artery and arteriole dilation impairment of $46 \pm 2\%$ and $46 \pm 8\%$ during low-level hypercapnia and $46 \pm 3\%$ and $45 \pm 2\%$ inhibition during high-level hypercapnia for genistein-pretreated animals. Both sets of these inhibition values are significantly different from the respective values in the absence of tyrphostin A23 or genistein. Administration of a higher concentration of genistein (10^{-5} M) produced no further protection of hypercapnic dilation postinsult compared with that obtained with genistein (10^{-6} M) (Fig. 3). On a percentage basis, these data reflect $49 \pm 5\%$ and $52 \pm 5\%$ inhibition during low-level hypercapnia and $44 \pm 3\%$ and $45 \pm 3\%$ inhibition during high-level hypercapnia, respectively.

In addition, pretreatment with either U-0126 (10^{-6} M) or PD-98059 (10^{-5} M) before insult also partially protected impairment of the hypercapnic dilation after hypoxia/ischemia (Fig. 4). On a percentage basis, these data reflect a pial small artery and arteriole dilation impairment of $47 \pm 6\%$ and $35 \pm 5\%$ during low-level hypercapnia and $50 \pm 6\%$ and $42 \pm 7\%$ inhibition during high-level hypercapnia for U-0126-pretreated

animals. These data also reflect a pial small artery and arteriole dilation impairment of $44 \pm 7\%$ and $31 \pm 5\%$ during low-level hypercapnia and $54 \pm 6\%$ and $39 \pm 7\%$ inhibition during high-level hypercapnia for PD-98059-pretreated animals. Both sets of these percent inhibition values are significantly different from the respective values in the absence of either U-0126 or PD-98059. Administration of a higher concentration of U-0126 (10^{-5} M) produced no further protection of hypercapnic dilation postinsult compared with that obtained with U-0126 (10^{-6} M) (Fig. 4). Combined administration of genistein (10^{-5} M) with U-0126 (10^{-5} M) also had no further significant protective effect.

Influence of PTK and MAPK inhibitors on pial artery diameter and hypercapnic pial artery dilation. Topical tyrphostin A23, genistein, U-0126, and PD-98059 all had no effect on pial artery diameter by themselves (118 ± 6 vs. 120 ± 6 μm and 121 ± 5 vs. 128 ± 6 μm before and after 10^{-6} and 10^{-5} M genistein, respectively). Similarly, all inhibitors had no effect on hypercapnic pial artery dilation in the absence of NOC/oFQ or hypoxia/ischemia (Fig. 5). However, combined genistein (10^{-5} M) and U-0126 (10^{-5} M) increased diameter (120 ± 5 vs. 147 ± 10 μm). Finally, there were no group differences in baseline diameter during normocapnia after hypoxia/ischemia.

Blood chemistry and mean arterial blood pressure. The blood chemistry and mean arterial blood pressure values were collected before and after all experiments and during periods of hypoxia and hypercapnia. Hypoxia decreased PO_2 to 34 ± 3 mmHg. Low hypercapnia

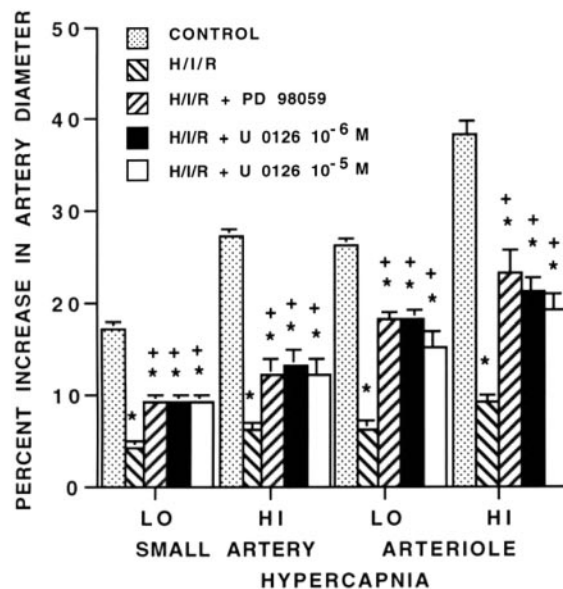


Fig. 4. Influence of low and high hypercapnia on pial small artery and arteriole diameter before (control), after hypoxia/ischemia-reperfusion (H/I/R), after hypoxia/ischemia in PD-98059 (10^{-5} M)-pretreated animals, after hypoxia/ischemia in U-0126 (10^{-6} M)-pretreated animals, and after hypoxia/ischemia in U-0126 (10^{-5} M)-pretreated animals; $n = 7$. * $P < 0.05$ compared with the corresponding control value; + $P < 0.05$ compared with the corresponding hypoxia/ischemia nonpretreated value.

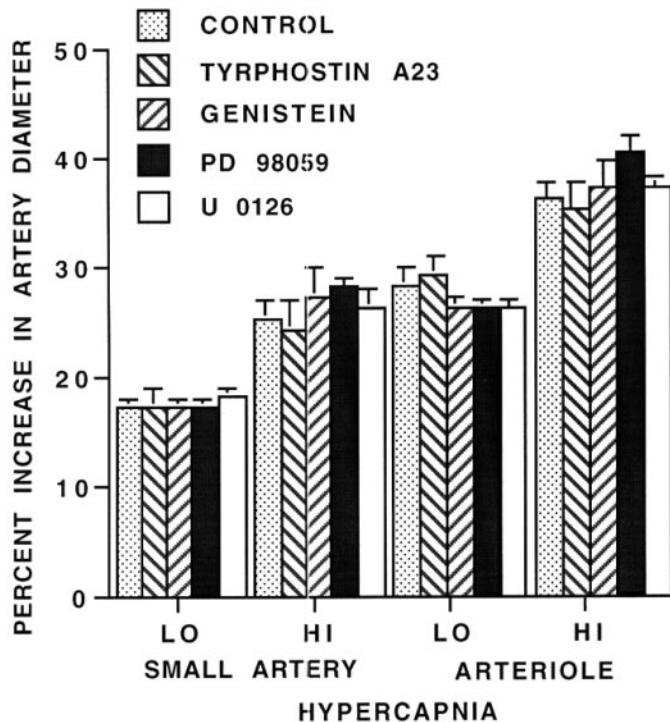


Fig. 5. Influence of low and high hypercapnia on pial small artery and arteriole diameter in the absence (control) and presence of either tyrphostin A23 (10^{-5} M), genistein (10^{-6} M), PD-98059 (10^{-5} M), or U-0126 (10^{-6} M); $n = 7$.

raised P_{CO_2} to 56 ± 2 mmHg, and high hypercapnia raised P_{CO_2} to 74 ± 3 mmHg. Carbon dioxide levels were kept constant during periods of hypoxia, and oxygen levels were kept constant during periods of hypercapnia. Before and after all experiments, the pH, P_{CO_2} , P_{O_2} , and mean blood pressure were unchanged.

DISCUSSION

The results of the present study show that coadministration of NOC/oFQ, in a concentration observed in cortical periarachnoid CSF after hypoxia/ischemia (10^{-10} M) (3), with hypercapnia attenuated pial artery vasodilation in response to this stimulus, consistent with earlier work (13). Because the concentration of NOC/oFQ had no effect on pial artery diameter by itself, decremented hypercapnic dilation did not result from physiological antagonism. These experiments were designed to biochemically mimic hypoxic/ischemic conditions regarding NOC/oFQ. These data suggest that such concentrations of this opioid-like peptide observed after hypoxia/ischemia could have physiological significance. Another series of experiments was then designed to determine the mechanism for such NOC/oFQ-induced impairment of hypercapnic dilation. For example, the data in the present study show that coadministration either of the PTK inhibitors tyrphostin A23 or genistein with NOC/oFQ partially prevented the inhibition of hypercapnic dilation under such biochemically mimicked injury conditions. Administration of genistein and administration of tyrphostin A23 alone had no effect on arterial diameter by itself, sug-

gesting a lack of physiological antagonism as a potential explanation for such observations. In addition, coadministration of each of the MAPK inhibitors U-0126 and PD-98059 with NOC/oFQ also partially prevented the inhibition of hypercapnic dilation. Similarly, administration of either U-0126 or PD-98059 alone had no effect on arterial diameter by itself. These data show that PTK and MAPK activation contributes to NOC/oFQ-induced impairment of hypercapnic dilation. The use of two structurally distinct PTK and MAPK inhibitors (8, 12) in the above study design strengthened such conclusions regarding the role of these two signal transduction pathways in the modulation of hypercapnic dilation by NOC/oFQ. Furthermore, the use of two concentrations of genistein and U-0126 (10^{-6} and 10^{-5} M) helped to establish that the lower concentration was near maximally efficacious in inhibition of PTK and MAPK, respectively.

The functional significance of the above-noted interaction of NOC/oFQ with hypercapnia has been investigated previously. The results of these studies show that hypoxia/ischemia blunted hypercapnia-induced pial artery dilation at 1 h of reperfusion (13), similar to previous studies that investigated the effects of ischemia-reperfusion without prior hypoxia on hypercapnic cerebrovasodilation (18). However, new data show that the NOC/oFQ receptor antagonist [F/G]NOC/oFQ(1-13)NH₂ partially prevented such diminished hypercapnic dilation postinsult (13). These data suggest the involvement of released NOC/oFQ after hypoxia/ischemia in altered hypercapnic cerebrovasodilation after this insult (13).

Accordingly, the present study was designed to characterize mechanisms involved in such a NOC/oFQ contribution to hypercapnic dilation impairment postinsult. The results of these studies show the pretreatment with genistein or tyrphostin A23 partially protected hypoxic/ischemic impairment of hypercapnic pial artery dilation. Because both genistein and tyrphostin are two structurally distinct inhibitors of PTK, these data suggest that activation of PTK is involved in the mechanism by which hypoxia/ischemia impairs hypercapnic dilation. Pretreatment with the structurally distinct MAPK inhibitors U-0126 or PD-98059 also partially protected hypoxic/ischemic impairment. These data suggest that activation of MAPK is also involved in the mechanism by which this insult impairs hypercapnic dilation. Taken together, these data further suggest that activation of PTK and MAPK is also involved in the mechanism by which NOC/oFQ impairs hypercapnic dilation after hypoxia/ischemia. Administration of higher concentrations (10^{-5} M) of genistein and U-0126 had no further protective effect, indicating that the lower concentration (10^{-6} M) was near maximal in efficacy. However, because either combined or single administration of such antagonists did not completely restore impaired hypercapnic dilation posthypoxia/ischemia, these data suggest that other mechanisms are involved in such impairment. Because MAPK is actually a family of kinases (ERK, JNK, and p38) and U-0126 and PD-98059 are thought to be more selective

ERK MAPK inhibitors, it is speculated that either JNK or p38 MAPK activation may also be contributory to such impairment. Alternatively, PTK and/or MAPK inhibitors may also have protective effects on neurons during ischemia. Thereby, they may have indirect effects on neuronal metabolism and function after reperfusion, which, in turn, would influence reactivity to hypercapnia.

Because the drugs used as probes for PTK and MAPK activation did not have any effect on pial artery diameter in sham control animals, these data indicate that these signal transduction pathways do not significantly contribute to the control of resting cerebrovascular tone. Additionally, such antagonists did not have any effect on hypercapnic dilation in the absence of hypoxia/ischemia or NOC/oFQ, indicating a greater role for such signal transduction pathways in pathological compared with physiological states. Such observations are consistent with the idea that PTK and MAPK are activated with injury, such as ischemia, and thereby contribute to cerebral vasospasm (14). The choice of concentration for the PTK and MAPK inhibitors used in the present study was based on in vitro assay data that demonstrate efficacy and selectivity (8, 12) as well as on in vivo data in the newborn pig (4).

Global cerebral ischemia in a piglet model has been previously observed to result in blunted pial artery dilation to hypercapnia (18). The cerebral vasodilation caused by hypercapnia and hypotension requires active prostaglandin synthesis, whereas dilation to isoproterenol does not (6, 16, 17, 30). After cerebral ischemia, elevated cerebral prostaglandin synthesis during hypercapnia and hypotension does not occur (18, 19). Vasodilation in response to these stimuli is likewise absent, whereas the responses to isoproterenol are unchanged (18, 19, 22). These studies suggest, then, that prostaglandin-associated stimuli are preferentially altered by cerebral ischemia. More recent studies have shown that one prostaglandin, PGI₂, subserves a permissive role in hypercapnic pial artery dilation (23), which, in turn, is modulated by PTK (27).

The mechanism for impaired hypercapnic cerebrovasodilation after cerebral ischemia has proven to be more elusive. Oxygen free radicals such as superoxide anion are released after cerebral ischemia (5) and are known to contribute to impaired vascular responsiveness postinsult. However, superoxide anion release does not contribute to impaired hypercapnic cerebrovasodilation after cerebral ischemia in the piglet (24). Inactivation of PGH synthase, like superoxide anion, does not appear to be involved, because conversion of exogenous arachidonic acid to prostanoids on the brain surface is not altered after cerebral ischemia (21). Therefore, ischemia may decrease the release of arachidonic acid in response to the specific induction stimuli. Inasmuch as phospholipase A₂ appears to be involved in the increase in prostanoid synthesis caused by hypercapnia in newborn pigs (30), it is possible that ischemia results in phospholipase A₂ inactivation. Another possibility that must be considered is that of depletion of arachidonic acid from a specific membrane

pool that provides a source for hypercapnia- and hypotension-induced arachidonic acid release. The marked increase in free arachidonic acid induced by ischemia (1, 31) makes such a possibility more attractive. Consistent with this hypothesis, topically applied arachidonic acid restores pial arteriolar dilation to hypercapnia after ischemia (22).

The results of the present study extend the above findings to indicate that a relatively newly described opioid, NOC/oFQ, contributes to impaired hypercapnic cerebrovasodilation after hypoxia/ischemia. Such an interaction is not entirely surprising in that opioids exert prominent effects in the control of the perinatal cerebral circulation, particularly under pathological conditions (2).

In conclusion, the results of the present study show that PTK and MAPK activation contribute to NOC/oFQ-induced impairment of hypercapnic dilation. These data suggest that activation of PTK and MAPK is also involved in the mechanism by which NOC/oFQ impairs hypercapnic dilation after hypoxia/ischemia.

The authors thank John Ross for excellent technical assistance in the performance of the experiments.

This research was supported by grants from the National Institutes of Health and the American Heart Association, Pennsylvania-Delaware Affiliate.

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