Low CO_2 Stimulates Inositol Phosphate Turnover and Increased Inositol 1,4,5-Trisphosphate Levels in Piglet Cerebral Microvascular Smooth Muscle Cells (43871)

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Abstract. **In contrast to hypercapnic dilation, hypocapnia-induced cerebral vasoconstriction does not involve prostanoids in newborn pigs. The hypothesis that increased pH or decreased CO_2 tension increases inositol phosphate turnover in piglet cerebral microvascular smooth muscle (SM) cells was addressed to begin to assess the possibility that this second-messenger system is involved in hypocapnia-induced cerebral vasoconstriction. Cerebral microvascular SM cells in primary culture prelabeled with [^{3}H]-myoinositol were stimulated for 30 sec with artificial cerebrospinal fluid of increased or normal pH, (7.80 vs 7.40), constant PCO_2 36 mm Hg. Following extraction from cells, radiolabeled inositol phosphates were separated by HPLC. These metabolic alkalosis studies were repeated using an inositol 1,4,5-trisphosphate (Ins[1,4,5]P_3 protein-binding assay (PBA). Respiratory alkalosis using aCSF with pH 7.60, PCO_2 20 mm Hg versus control pH 7.40, PCO_2 36 mm Hg was similarly tested with PBS measurement of Ins(1,4,5)P_3. aCSFs of control pH 7.40, and PCO_2s of 70, 36, or 25 mm Hg were studied both by [^{3}H]-myoinositol (HPLC) and PBA to further determine the importance of CO_2 tension, in the presence of fixed pH, on Ins(1,4,5)P_3 production. When PCO_2 was constant, inositol phosphate turnover (as measured by [^{3}H]-Ins[1,4,5]P_3 accumulation) increased when pH was increased from 7.40 to 7.80 at 30 sec of stimulation. Mean [^{3}H]-Ins(1,4,5)P_3 accumulation at pHs of 7.40 and 7.80, constant PCO_2 of 36 mm Hg, were 2.9 ± 0.7 and 4.1 ± 0.8 cpm/μg protein, respectively. Ins(1,4,5)P_3 levels for pH of 7.40 or 7.80 and constant PCO_2 of 36 mm Hg, were 25.4 ± 1.8 and 38 ± 8 pmol/well, respectively, by PBA. Respiratory alkalosis also increased Ins(1,4,5)P_3 levels. For pH of 7.40, PCO_2 36 mm Hg and pH 7.60, PCO_2 20 mm Hg, Ins(1,4,5)P_3 levels were 37.6 ± 16 and 64.1 ± 25 pmol/well, respectively. Decreasing CO_2 tension (from 70 mm Hg to 25 mm Hg) in the presence of fixed pH 7.40 failed to increase Ins(1,4,5)P_3 levels. The present data demonstrate that decreased CO_2 tension stimulates an increase in Ins(1,4,5)P_3 production in piglet cerebral microvascular smooth muscle cells. Increasing pH via lower PCO_2 increases the level of Ins(1,4,5)P_3 even more than increasing pH with fixed base, but extracellular pH appears to be important since decreased PCO_2 without changing extracellular pH had no effect. We conclude that the inositol phosphate second messenger system in cerebral microvascular smooth muscle responds appropriately to acute alkalosis to be involved in hypocapnia-induced cerebral vasoconstriction.** [P.S.E.B.M. 1995, Vol 209]

Arterial CO_2 tension has marked effects on cerebral blood flow in adult and immature animals. In newborn pigs, hypercapnia produces intense cerebral vasodilation via a mechanism that involves prostanoids and is blocked by indomethacin (11, 14, 15, 24). Primary cultures of cerebral microvascular endothelial cells, but not smooth muscle or glia, produce vasodilatory prostanoids in response to hypercapnia (8). Hypocapnia induced cerebral vasocon-

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striction, on the other hand, appears to occur via a prostanoid independent mechanism as indomethacin effects no change in cerebral arteriolar constriction in response to hypocapnia (15).

Hyperventilation to produce hypocapnia is employed in a variety of disease states to alter organ blood flow. For example, in newborns and children suffering traumatic brain injuries, hyperventilation is used to attempt to decrease cerebral blood flow and avoid deleterious increases in intracranial pressure. In the pulmonary vascular bed, hyperventilation can decrease pulmonary vascular resistance and facilitate oxygen loading to hemoglobin. What effects hypocapnia may ultimately have on the immature cerebral circulation cannot be understood without first exploring the mechanisms leading to an increase in vascular tone. To date, the mechanism(s) affecting an increase in cerebral vascular tone from hypocapnia remains elusive. It seems very plausible that an increase in pH triggers the calcium mobilizing inositol 1,4,5-trisphosphate (Ins[1,4,5]P_3) second-messenger system to produce an increase in tone. To date, however, there is no evidence that the Ins(1,4,5)P_3 second messenger system is activated in response to an increase in pH or decrease in CO_2 tension in piglet cerebral microvascular smooth muscle (SM) cells. Mechanistically, a decrease in extracellular H^+ concentration may affect a membrane protein that is coupled to a phosphoinositide-specific phospholipase C capable of generating the Ca^{2+}-mobilizing second messenger Ins(1,4,5)P_3 with resultant cerebral vasoconstriction. The ensuing studies were therefore carried out to address the hypothesis that decreased PCO_2 stimulates inositol phosphate turnover, increasing Ins(1,4,5)P_3 levels in piglet cerebral microvascular smooth muscle cells.

Materials and Methods

All procedures involving animals were reviewed and approved by the Animal Care and Use Committee at the University of Tennessee, Memphis, TN. All procedures were done using sterile technique.

Chemicals and Supplies. Fetal bovine serum was purchased from Hyclone (Logan, UT). Vitrogen 100 was from Collagen (Palo Alto, CA). All other chemicals or biochemicals were purchased from Sigma Chemical Co. (St. Louis, MO). Disposable culture plates were from Costar (Cambridge, MA). Tissue culture plates were from Becton Dickinson Labware (Lincoln Park, NJ). Nylon mesh screens were obtained from Spectrum (Houston, TX). [^{3}H]-myoinositol and inositol mono-, bis-, and 1,4,5-trisphosphate standards were obtained from American Radiolabeled Chemicals, Inc. (St. Louis, MO). Anion Sep-Pak exchange cartridges used for separation of the inositol phosphates were obtained from Waters Chromatography Division/Millipore Corporation (Milford, MA). D-myo-Inositol 1,4,5-trisphosphate (IP_3) [^{3}H] protein-binding assay system kits were obtained from Amersham (Amersham, UK).

Isolation and Culture of Microvascular Smooth Muscle Cells from Newborn Pig Brain. Brains were removed from newborn pigs 1 to 3 days old. Cerebral microvascular smooth muscle cells were prepared as described previously (8). Newborn pig brain cortex (20 g) was removed under ketamine hydrochloride (33 mg/kg im) and acepromazine (3.3 mg/kg im) anesthesia, placed into a beaker with 40 ml cold isolation solution containing medium 199 (M199), 0.015 *M* N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid, 1 U/ml sodium heparin, and antibiotic-antimycotic solution (100 U/ml penicillin, 100 μg/ml streptomycin, and 2.5 μg/ml amphotericin B). The meninges and big vessels were removed from the tissue, and the tissue was washed three times with M199 isolation solution. The tissue was minced into tiny pieces using two scalpels in 20 ml M199 isolation solution and then transferred to a 40-ml Dounce homogenizer and homogenized with 10 strokes of loose-fitting pestle. The homogenate was passed through a 300-μm nylon mesh screen. The passage was refiltered over a 60-μm nylon mesh screen. The screen was removed and placed in a 50-ml centrifuge tube containing 50 ml of M199 isolation solution. The microvessels (60–300 μm) were washed off by agitation and scraping, and then centrifuged at 500*g* for 5 min. Isolated microvessels were directly placed on collagen-coated plastic surfaces in 5-ml Becton Dickinson Labware plates in DMEM(SM) containing 20% FBS, 2 mg/ml sodium bicarbonate, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2.5 μg/ml amphotericin B. Cultures were maintained in a 5% CO_2 air incubator at 37°C. Smooth muscle cells grew out from the ends of adherent microvessels. The first media change occurred 72 hr later to remove adherent and nonadherent vessels with smooth muscle cells that grew out from these vessels left behind. Subsequently, the media was changed every 2 to 3 days. Experiments were performed after 14–21 days of cultivation. Prior characterization of primary microvascular smooth muscle cultures were ~95% vascular smooth muscle (α-smooth muscle actin) with ~4% glia (GFAP) and 1% endothelial cells (factor VIII) (4, 8, 9).

Experimental Design. Prior to experimentation, cells were incubated with 25 μCi[^{3}H]-myoinositol in myoinositol free medium (RPMI-1640) for 24 hr (37°C, 5% CO_2). Incorporation of [^{3}H]-myoinositol and the general procedures for applying and stopping treatments were modified from Homayoun and Harik (6). On the day of experimentation, cells were washed twice and then incubated for 1 hr at 37°C, 5% CO_2 in control artificial cerebrospinal fluid (aCSF) (KCl 3.0

mM, $MgCl_2$ 0.6 mM, $CaCl_2$ 2.0 mM, glucose 3.7 mM, urea 6.7 mM, NaCl 127 mM, $NaHCO_3$ 27.4 mM; pH 7.40, PCO_2 36). aCSF for the metabolic alkalosis pH 7.80, PCO_2 36 mm Hg versus control pH 7.40, PCO_2 36 mm Hg studies contained differing amounts of NaCl and $NaHCO_3$ in accordance with the Henderson-Hasselbach equation. For aCSF of pH 7.80, PCO_2 36 mm Hg, there was 97 mM NaCl and 60 mM $NaHCO_3$. Each of these solutions was bubbled for 1 to 2 hr with 5% CO_2 at 37°C. For the respiratory alkalosis versus control aCSF studies, a solution of initial pH 7.40, PCO_2 36 mm Hg was bubbled for 1 to 2 hr with 2.5% CO_2, 37°C to achieve a pH of 7.6, PCO_2 20 mm Hg ($NaHCO_3$ 27.4 mM). The pH and PCO_2 of all solutions were measured before addition to the cells. The cells were incubated under the appropriate CO_2 atmosphere. After treatment with lithium (50 mM) for 1 min, each well of cells was stimulated with control or test aCSF for 30 sec in the appropriate CO_2 atmosphere. Lithium was used to inhibit enzymes involved in hydrolysis of inositol phosphates, thus causing accumulation of desired inositol phosphate species.

Determination of Inositol Phosphates by High-Performance Liquid Chromatography. The reactions were terminated by rapid aspiration of media and addition of methanol:HCL (2:0.05). Cells were homogenized in chloroform:methanol:HCL (1:2:0.05). Following centrifugation at 15,000*g* for 15 min at 4°C, the supernatant was evaporated under N_2. Protein in the pellet was determined using the Lowry method. Following evaporation of the supernatant, the residue was suspended in 10 ml of water and placed on a Waters Accell plus QMA cartridge (anion exchange) prewashed with water. The cartridges were washed with an additional 4 ml of water and the inositol phosphates eluted with 2 ml of 1.0 *M* ammonium phosphate. 0.5 ml samples of these eluents were injected directly into the HPLC. HPLC was performed on a 25 cm × 4.6 mm Partisil 10 SAX column with isocratic perfusion at 2.0 ml/min with water, pH 2.7 for 2.5 min, 0.35 *M* ammonium phosphate, pH 3.5 for 7.5 min, and 1.5 *M* ammonium phosphate, pH 3.5, for 15 min. On line detection of radioactivity eluting from the column was accomplished with a flo-one β radioactive flow detector.

Determination of Ins(1,4,5)P_3 by Protein-Binding Assay. After treatment with lithium (50 mM) for 1 min, each well of cells was stimulated with 900 μl of control or test aCSF for 30 sec in the appropriate CO_2 atmosphere. The reactions were terminated by addition of 1.0 *N* HCL. Cells were sonicated for 20 sec and then centrifuged at 15,000 *g* for 20 min at 4°C. The supernatant was used for quantitative determinations of Ins(1,4,5)P_3 with a D-myo-inositol 1,4,5-trisphosphate (IP_3) [^{3}H] protein-binding assay system assembled by Amersham International. Since an acid extraction method was used, each sample was neutralized with 1.0 *N* Tris base to achieve a pH between 7.5 and 8.5 before performing the PBA.

Statistical Analysis. Data are presented as means ± SEM from three to eight experiments per group. Statistical analysis was performed using analysis of variance and a post hoc Tukey's protected *t* test for planned comparisons. Values of $P \leq 0.05$ were regarded as significant.

Results

The bottom tracing in Figure 1 shows the resolution of myo-inositol and standard inositol mono-, bis-, and 1,4,5-trisphosphate achieved in 25 min on the Partisil 10 SAX HPLC column. This HPLC methodology was used to begin to assess inositol phosphate turnover in our cell system. There is no prior data on newborn piglet cerebral microvascular smooth muscle cells and inositol phosphate turnover. The top tracing

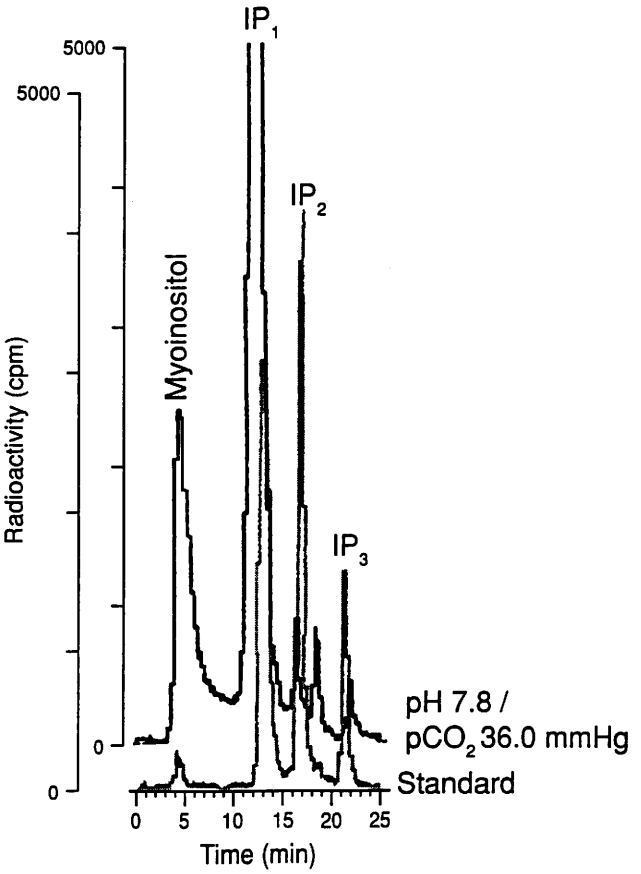

Figure 1. HPLC resolution of [^{3}H]-myoinositol and the three [^{3}H]-inositol phosphate standards (bottom tracing). Top tracing—representative spectra of cerebral microvascular smooth muscle cells stimulated 30 sec with aCSF of pH 7.80, PCO_2 36 mm Hg. Perfusion occurred at 2 ml/min with water (pH 2.7), 0.35 *M*, and 1.5 *M* ammonium phosphate (pH 3.5). Elution of inositol phosphates was achieved in 25 min on the Partisil 10 SAX column (25 cm × 4 mm). IP_3 is Ins(1,4,5)P_3. On-line detection of radioactivity eluting from the column was accomplished by a flo-one β radioactive flow detector.

of the figure represents a typical spectra of cerebral microvascular smooth muscle cells stimulated for 30 sec with test aCSF of pH 7.80, PCO_2 36.0 mm Hg. IP_3 is Ins(1,4,5)P_3.

Effect of Increased pH, Normal CO_2 Tension (Metabolic Alkalosis) on Cerebral Microvascular Smooth Muscle Cells. Increased inositol phosphate turnover occurred in response to a metabolic alkalosis stimulus (pH 7.80, PCO_2 36 mm Hg) such that the mean [^{3}H]-Ins(1,4,5)P_3 peak at 30 sec was 4.1 ± 0.8 cpm/μg protein compared with the control stimulus, pH 7.40, PCO_2 36 mm Hg [^{3}H]-Ins(1,4,5)P_3 peak of 2.85 ± 0.6 cpm/μg protein (Fig. 2). These experiments were also performed using an Ins(1,4,5)P_3 PBA. The metabolic alkalosis versus control aCSF stimuli produced Ins(1,4,5)P_3 levels of 38 ± 8 pmol/well versus 25.4 ± 1.8 pmol/well, respectively (Fig. 3). Also of note is that decreasing CO_2 tensions (70, 36, 25, and 10 mm Hg) at constant pH, did not significantly increase mean [^{3}H]-Ins(1,4,5)P_3 peak at 30 sec (1.43 ± 0.21, 1.30 ± 0.23, 1.57 ± 0.21, and 1.33 ± 0.25 cpm/μg protein, respectively).

Effect of Increased pH, Decreased CO_2 Tension (Respiratory Alkalosis) on Cerebral Microvascular Smooth Muscle Cells. A 30-sec stimulus of respiratory alkalosis, pH 7.6, PCO_2 20 mm Hg compared with control pH 7.40, PCO_2 36 mm Hg, produced Ins(1,4,5)P_3 levels of 64.1 ± 25 pmol/well versus 37.6 ± 16 pmol/well, respectively (Fig. 4). These data demonstrate that decreased CO_2 tension stimulates inositol phosphate turnover (as detected by HPLC) and Ins(1,4,5)P_3 levels (as detected by PBA) in piglet cerebral microvascular smooth muscle cells. The increase in pH from low CO_2 (pure respiratory alkalosis) increased Ins(1,4,5)P_3 levels to a greater extent than an increase in pH from fixed base (metabolic alkalosis).

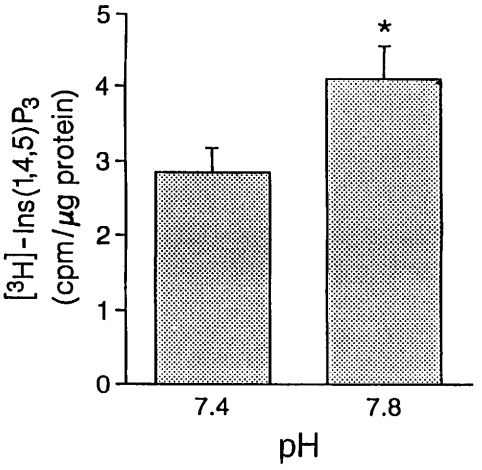

Figure 2. Effect of increased pH, normal CO_2 tension (metabolic alkalosis) on [^{3}H]-Ins(1,4,5)P_3 accumulation in cerebral microvascular smooth muscle cells by HPLC. Stimulation time: 30 sec. Values are mean ± SEM. $^*P \leq 0.05$. cpm = counts per minute.

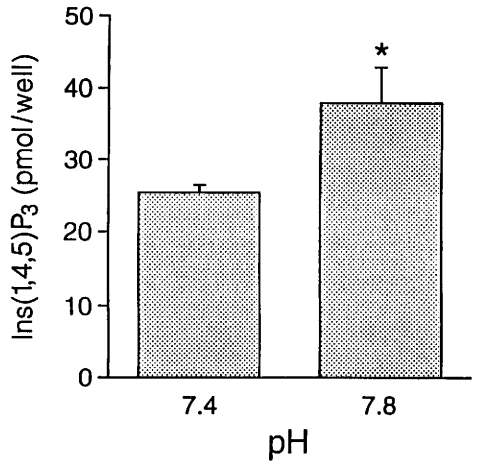

Figure 3. Effect of increased pH, normal CO_2 tension (metabolic alkalosis) on Ins(1,4,5)P_3 level in cerebral microvascular smooth muscle cells by PBA. Stimulation time: 30 sec. Values are mean ± SEM. $^*P \leq 0.05$.

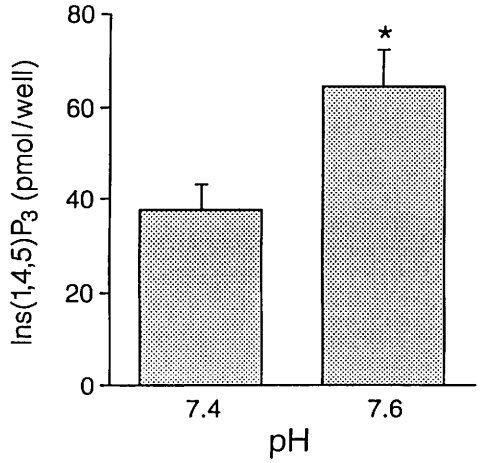

Figure 4. Effect of increased pH, decreased CO_2 tension (respiratory alkalosis) on Ins(1,4,5)P_3 in cerebral microvascular smooth muscle cells by PBA. Stimulation time: 30 sec. Values are mean ± SEM. $^*P \leq 0.05$.

Discussion

The present data demonstrate that metabolic alkalosis and respiratory alkalosis increase inositol phosphate turnover increasing the level of Ins(1,4,5)P_3 in cultured newborn piglet cerebral microvascular smooth muscle cells. The increase in pH was an important factor in increasing the level of this second messenger, since normal pH with pCO_2 20 mm Hg did not produce an increase in Ins(1,4,5)P_3.

The cellular mechanisms by which $PaCO_2$ influences the cerebral circulation remain unclear, especially in light of the different vascular responses seen when CO_2 is above or below the physiological level. With hypercapnia, for example, cyclic nucleotides are involved in cerebral vasodilation via a mechanism involving prostanoids in newborn pigs (19). These vasodilatory prostanoids are of microvascular endothe-

lial cell origin in the piglet cerebral circulation (8). Light/dye injury with sodium fluorescein produces selective damage of these endothelial cells with loss of production of vasodilatory prostanoids and vasodilation to hypercapnia (12). Cerebral vasoconstriction, on the other hand, does not involve prostanoid-dependent mechanisms. Indomethacin does not block cerebral vasoconstriction in the presence of hypocapnia in newborn pigs (15). An unidentified vasoconstrictor mechanism(s) is invoked during hypocapnic vasoconstriction that relates to the absolute PCO_2 and not the direction of change. Therefore, it is conceivable that a second messenger causing constriction is progressively activated as PCO_2 is decreased below "physiological baseline" but is not affected by deviations of CO_2 above that level. An increase in pH via a decrease in CO_2 tension may act directly on cerebral microvascular smooth muscle cells via alternate second messenger systems, such as Ins(1,4,5)P_3, capable of increasing cytosolic calcium and, thereby, increasing tone.

In this study, aCSF of decreasing CO_2 concentrations and normal pH 7.40, did not stimulate an increase in Ins(1,4,5)P_3 compared with aCSF of increased pH, normal CO_2 tension (metabolic alkalosis), or increased pH, low CO_2 (respiratory alkalosis). Our solutions were continuously bubbled with the desired partial pressure of PCO_2, and the pH and PCO_2 of these solutions were checked prior to stimulation of the cells. Our aCSFs always contained CO_2/HCO_3^- essential for maintenance of the microvascular smooth muscle cell pumps such as the sodium-independent Cl^--HCO_3^- exchanger. The activity of this pump is critical to recovery from an alkaline load and normal cell homeostasis (1, 10, 18). We found that an increased pH of 7.80 with constant PCO_2 (metabolic alkalosis) or pH of 7.60 with PCO_2 of 20 mm Hg (pure respiratory alkalosis) increased Ins(1,4,5)P_3 levels in our cerebral microvascular smooth muscle cells.

From this study, it appears that an important factor in stimulating the rise in Ins(1,4,5)P_3 is the decrease in H^+ ion concentration. However, a pH of 7.6 from CO_2 in the respiratory alkalosis studies produced a greater increase in Ins(1,4,5)P_3 levels than pH of 7.8 from fixed base in the metabolic alkalosis studies. These data suggest the importance of increased extracellular pH (pH_e) on inositol phosphate turnover. Decreased CO_2 tensions failed to increase Ins(1,4,5)P_3 accumulation in the presence of a normal pH_e. The contribution of intracellular pH (pH_i) in response to pH_e, which would be more affected by respiratory than metabolic alkalosis, or a contribution of CO_2 independent of pH cannot be ruled out in this study.

While a few studies, in a variety of cell types, explain mechanisms whereby Ins(1,4,5)P_3-induced Ca^{2+} mobilization may be due to or enhanced by alkalinization, most focus on the effect of this mobilization from the increased pH_i not pH_e standpoint. For example, acute intracellular alkalinization may affect an increase in Ins(1,4,5)P_3, and also enhance the sensitivity of the sarcoplasmic reticulum to Ins(1,4,5)P_3 as has been demonstrated in platelets (2). In addition, intracellular alkalinization may cause Ins(1,4,5)P_3 receptors to become sensitized to endogenous levels of Ins(1,4,5)P_3 (16). Specifically, an increase in pH_i may cause Ins(1,4,5)P_3-sensitive stores to release their Ca^{2+} when overloaded with Ca^{2+} through a mechanism that requires a threshold level of Ins(1,4,5)P_3 and can be sensitized by sulphydryl reagents (16). In some systems, such as Purkinje cells, the highest binding level of Ins(1,4,5)P_3 to cerebellar membranes occurs in the alkaline pH_i range (22). Such increases in Ins(1,4,5)P_3 sensitivity or binding could increase $[Ca^{2+}]_c$ important for producing increasing vascular tone. There is also some evidence to suggest that Ins(1,4,5)P_3-sensitive nonmitochondrial Ca^{2+} pools may be pH_i regulated and important in modulating intracellular free Ca^{2+} concentrations (21). The hypothesis and results of this study, however, suggest that an extracellular pH trigger is important in mobilizing the Ins(1,4,5)P_3 second messenger system. We propose that a decrease in the H^+ ion concentration may affect a membrane protein coupled with a phosphoinositide specific phospholipase C capable of generating the Ca^{2+} mobilizing second messenger Ins(1,4,5)P_3. While the studies presented here did not investigate the effects of pH_e on pH_i, other investigators in our laboratory have shown that pH_e changes, even with constant PCO_2, also affect pH_i (8). Increasing pH_e via respiratory alkalosis would increase pH_i more, and more rapidly, than would that via metabolic alkalosis. Once Ins(1,4,5)P_3 is activated by the extracellular pH trigger, however, it may trigger further calcium release via calcium-induced calcium release mechanisms in mitochondria and other intracellular calcium stores (20, 25).

With our current methodology, we cannot rule out the possibility that even larger increases in the Ins(1,4,5)P_3 levels would have occurred if we had measurements prior to 30 sec of stimulation. There are studies to suggest that in some cell types peaks in Ins(1,4,5)P_3 levels may occur as early as 3 to 10 sec (23). For example, norepinephrine will dramatically increase Ins(1,4,5)P_3 within 5 to 10 sec in vascular smooth muscle of rat tail artery. This effect is markedly diminished by 20 sec (7). We did not employ shorter stimulation times given the possibility of introducing significant errors in technique in our experimental methodology. However, even at 30 sec the inositol phosphate second messenger system was significantly stimulated.

This study necessitates the discovery of other mechanisms to explain how hypocapnia, in the pres-

ence of fixed pH, may cause an increase in cerebral vascular tone. While the data are far from conclusive, some studies suggest that other signal-activated phospholipases such as PLA_2 may be pH dependent and possibly capable of crosstalk with other membrane phospholipases (3, 13, 17). In such a case, it seems plausible that signal crosstalk may be involved in hypocapnic vasoconstriction or perhaps even have a modulatory role on $Ins(1,4,5)P_3$ levels. Yet other non-signal transduction mechanisms exist that may explain hypocapnic-mediated cerebral vasoconstriction. For example, in middle cerebral artery strips from cats, decreases in PCO_2 from physiological levels produce smooth muscle cell depolarization by reducing outward K^+ conductance (5). Such depolarization leads to contraction. However, this is the first study to show that the important signal transduction calcium-mobilizing second messenger, $Ins(1,4,5)P_3$, can be activated by increased pH from metabolic alkalosis and respiratory alkalosis in cerebral microvascular smooth muscle.

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1. Battle DC, Peces R, LaPointe MS, Ye M, Daugirdas JT. Cytosolic free calcium regulation in response to acute changes in intracellular pH in vascular smooth muscle. Am J Physiol **264**(Cell Physiol 33):C932–C943, 1993.
2. Brass LF, Joseph SK. A role for inositol trisphosphate in intracellular Ca^{2+} mobilization and granule secretion in platelets. J Biol Chem **260**:15172–15179, 1985.
3. Dennis EA. Diversity of group types, regulation, and function of phospholipase A_2. J Biochem **269**:13057–13060, 1994.
4. Gown AM, Vogel AM. Monoclonal antibodies to intermediate filament proteins of human cells: Unique and cross-reacting antibodies. J Cell Biol **95**:414–424, 1982.
5. Harder DR. Effect of H^+ and elevated PCO_2 on membrane electrical properties of rat cerebral arteries. Pfluegers Arch **394**:182–185, 1982.
6. Homayoun P, Harik SI. Bradykinin Receptors of cerebral microvessels stimulate phosphoinositide turnover. J Cereb Blood Flow Metab **11**:557–566, 1991.
7. Hong G, Martin H, Barsotti RJ, Labelle EF. Rapid increase in inositol phosphate levels in norepinephrine-stimulated vascular smooth muscle. Am J Physiol **261**(Cell Physiol 30):C17–C22, 1991.
8. Hsu P, Shibata M, Leffler CW. Prostanoid synthesis in response to high CO_2 in newborn pig brain microvascular endothelial cells. Am J Physiol **264**(Heart Circ Physiol 33):H1485–H1492, 1993.
9. Jaffe EA, Hoyer LW, Nanchman RL. Synthesis of antihemophilic factor antigen by cultured human endothelial cells. J Clin Invest **52**:2757–2764, 1973.
10. Kikeri D, Zeidel ML, Ballerman BJ, Brenner BM, Herbert SC. pH regulation and response to AVP in A10 cells differ markedly in the presence vs absence of CO_2-HCO_3^-. Am J Physiol **259**(Cell Physiol 28):C471–C483, 1993.
11. Leffler CW, Busija DW. Prostanoids in cortical subarachnoid cerebrospinal fluid and pial artery diameter in newborn pigs. Circ Res **57**:689–694, 1985.
12. Leffler CW, Mirro R, Shanklin DR, Armstead WM, Shibata M. Light/dye microvascular injury selectively eliminates hypercapnia-induced pial arteriolar dilation in newborn pigs. Am J Physiol **266**(Heart Circ Physiol 35):H623–H630, 1994.
13. Liscovitch M. Crosstalk among multiple signal-activated phospholipases. Trends Biochem Sci **17**:393–399, 1992.
14. Mirro R, Busija DW, Armstead WM, Leffler CW. Histamine dilates pial arterioles of newborn pigs through prostanoid production. Am J Physiol **254**(Heart Circ Physiol 23):H1023–H1026, 1988.
15. Mirro R, Pharris LJ, Armstead WM, Shibata M, Leffler CW. Effects of indomethacin on newborn pig pial arteriolar responses to PCO_2. J Appl Physiol **75**(3):1300–1305, 1993.
16. Missiaen L, Taylor CW, Berridge MJ. Spontaneous calcium release from inositol trisphosphate-sensitive calcium stores. Nature **352**:241–244, 1991.
17. Myatt EA, Stevens FJ, Sigler PB. Effects of pH and calcium ion on self-association properties of two dimeric phospholipases A_2. J Biol Chem **266**:16331–16335, 1991.
18. Neylon CB, Little PJ, Cragoe EJ Jr., Bobik A. Intracellular pH in human arterial smooth muscle. Circ Res **67**:814–825, 1990.
19. Parfenova H, Shibata M, Zuckerman LS, Mirro R, Leffler CW. Cyclic nucleotides and cerebrovascular tone in newborn pigs. Am J Physiol **265**(Heart Circ Physiol 34):H1972–H1982, 1993.
20. Sachs G, Muallem S. Sites and mechanisms of Ca^{2+} movement in non-excitable cells. Cell Calcium **10**:265–273, 1989.
21. Schulz I, Thévenod F, Dehlinger-Kremer M. Modulation of intracellular free Ca^{2+} concentration by IP_3-sensitive and IP_3-insensitive nonmitochondrial Ca^{2+} pools. **10**:325–336, 1989.
22. Snyder SH, Suppatopone S. Isolation and functional characterization of an inositol trisphosphate from brain. Cell Calcium **10**:337–342, 1989.
23. Stryjek-Kaminska D, Piiper A. Role of guanine nucleotides in hormone-stimulated inositol 1,4,5-trisphosphate production in pancreatic acini. DuPont Biotech **Update Fall**:59–61, 1993.
24. Wagerle LC, Mishra OP. Mechanisms of CO_2 response in cerebral arteries of the newborn pig: role of phospholipase, cyclooxygenase, and lipoxygenase pathways. Circ Res **62**:1019–1026, 1988.
25. Wakui M, Osipchuk YV, Peterson OH. Receptor-activated cytoplasmic Ca^{2+} spiking mediated by inositol trisphospate is due to Ca^{2+}-induced Ca^{2+} Release. Cell **63**:1025–1032, 1990.