

Effects of acid-base imbalance on vascular reactivity

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Acid-base homeostasis maintains systemic arterial pH within a narrow range. Whereas the normal range of pH for clinical laboratories is 7.35-7.45, *in vivo* pH is maintained within a much narrower range. In clinical and experimental settings, blood pH can vary in response to respiratory or renal impairment. This altered pH promotes changes in vascular smooth muscle tone with impact on circulation and blood pressure control. Changes in pH can be divided into those occurring in the extracellular space (pH_o) and those occurring within the intracellular space (pH_i), although, extracellular and intracellular compartments influence each other. Consistent with the multiple events involved in the changes in tone produced by altered pH_o , including type of vascular bed, several factors and mechanisms, in addition to hydrogen ion concentration, have been suggested to be involved. The scientific literature has many reports concerning acid-base balance and endothelium function, but these concepts are not clear about acid-base disorders and their relations with the three known mechanisms of endothelium-dependent vascular reactivity: nitric oxide (NO/cGMP-dependent), prostacyclin (PGI_2 /cAMP-dependent) and hyperpolarization. During the last decades, many studies have been published and have given rise to confronting data on acid-base disorder and endothelial function. Therefore, the main proposal of this review is to provide a critical analysis of the state of art and incentivate researchers to develop more studies about these issues.

Key words: Acid-base balance; Nitric oxide; Vascular reactivity; Endothelium; Alkalosis; Acidosis

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Introduction

Acid-base homeostasis maintains systemic arterial pH within a narrow range. Whereas the normal range of pH for clinical laboratories is 7.35-7.45, *in vivo* pH is maintained within a much narrower range. This degree of tight regulation is accomplished by chemical buffering in extracellular and intracellular fluids and regulatory responses that are controlled by the respiratory and renal systems. Changes in pH can have profound, yet poorly understood, influence on pulmonary and systemic vascular resistance and reactivity to pressor agents.

Changes in pH can be divided into those occurring in the extracellular space (pH_o) and those occurring within the intracellular space (pH_i), although, extracellular and

intracellular compartments influence each other. Cell studies have shown that changes in pH_o cause only small and slow changes in pH_i ; as a result, pH_o and pH_i have usually been considered separately (1). For example, in cardiac myocytes the change in pH_i was only around 30% of the pH_o change and occurred slowly (10-40 min) (2,3). However, in rat mesenteric resistance vessels the situation is different; changes in pH_o cause large (70% pH_o changes) and rapid (<2 min) changes in pH_i (4). Therefore, it appears that not all vascular smooth muscles behave in a similar manner when pH_o is changed, which may be a consequence of differing permeabilities to protons or mechanisms of regulating pH_i .

Consistent with the multiple events involved in the changes in tone produced by altered pH_o , including type of

vascular bed, several factors and mechanisms, in addition to hydrogen ion concentration, have been suggested to be involved. Prostanoids, purines, sensory neuro-transmitters (5-7), hyperpolarization, and changes in intracellular calcium concentration ($[Ca^{2+}]_i$) are candidates as well as the recently demonstrated vasoactive molecule nitric oxide (NO) (5). The most common manner to study NO action has been to interfere with the enzymatic production of this gas and the same experimental approach has been used in studies involving NO and metabolic vasodilation (8-10).

There are many reports concerning acid-base imbalance and endothelium function, but these concepts are, in general, too specific. The effects of pH and its mechanism of action may be expected to vary between vessel types. The effects of pH may alter with time or separate acute and chronic pathways may be evoked. The endothelium may well activate a different pathway from that of the smooth muscle and extracellular matrix and there is extensive literature on the important role played by the endothelium in mediating vascular responsiveness. This review analyzes how vascular tone can be regulated by endothelial and smooth muscle function under the influence of changes of pH.

Effect of pH_o on pH_i

In vitro studies about the measurement of pH_o are generally simple, occurring in perfusate; however, the measurement of pH_i is problematic and publications did not appear until the 1980's. This was because there was no suitable technique for measuring pH_i in small, contractile preparations. Presently, two main techniques are available: magnetic resonance and fluorescent indicators (using 2'7'-bis-carboxyethyl-5(6)-carboxyfluorescein (BCECF) and 5-(and-6)-carboxy SNARF[®]-1, acetoxymethyl ester, acetate).

Low pH values induce vessel dilation. When extracellular pH is 7.4, the corresponding intracellular value is 7.1 to 7.2 when actually it should be 6.4. This implies that protons are actively extruded from the cells against an electrochemical gradient (11-13). The main proton transporters are the Na^+/H^+ exchanger and the sodium coupled bicarbonate. The type 1 Na^+/H^+ isoform is localized in the vascular smooth muscle and can be activated by vasoconstrictors and growth factors, glucose, and hyperosmotic stress leading to increased rate of acid recovery, increased influx of sodium and restoration of cell volume. It is likely that intracellular concentration of calcium modulates the activation of this transporter, as well as a mechanism dependent on or independent of protein kinase C. Like-

wise, cGMP has also been implicated in the control of the exchange activity as a result of pH alterations. Unlike the Na^+/H^+ transporter, the Na^+/HCO_3^- exchanger may vary between smooth muscles. It appears that this exchanger has a plethora of different expressions and the exact physiological importance of each is challenging. The consensus is that this transporter accounts for a significant role in the control of pH_i , except for the guinea pig femoral artery where it has a very small fraction of acid extrusion. Additionally, there is a very consistent finding that smooth muscles have an Na^+ -independent Cl^-/HCO_3^- exchange pathway that has been shown in cultured and intact vascular preparations (14-16).

It is possible that the response to low pH_o values is mediated through the decrease in pH_i . Furthermore, most intracellular biochemical reactions of excitation-contraction coupling are potentially affected by low pH_i and often in the direction that favors relaxation. On the other hand, in most vascular beds, the immediate response to a selective decrease in pH_i is increased tension and some may respond with acute alkalinization, which may cause paradoxical relaxation of an agonist-induced response or have little effect on vascular tone. Thus, reduction of extracellular pH is probably more important for vasodilation than the associated reduction in pH_i . It is becoming clear that the reduction of $[Ca^{2+}]_i$ is involved significantly, although the role of membrane potential is not yet understood (14).

Control of vascular smooth muscle by external stimuli involves the generation of intracellular H^+ signals, which are transduced by the concerted action of a variety of cellular H^+ sensors. Intracellular pH sensors form signal complexes, which are able to convert H^+ signals to intracellular Ca^{2+} signals thereby enabling both homeostatic responses to acidosis and alkalosis as well as control of smooth muscle function (17).

Respiratory acidosis

The pH has marked effects on the blood flow of several vascular beds but the underlying mechanisms are incompletely understood. It is still not agreed, for example, whether it is the fall in pH_o or pH_i that is responsible for changes in tone resulting from hypercapnic acidosis. This issue has been further complicated by the recent discovery that NO may also be involved in vasodilator responses to hypercapnia. This finding has led some laboratories to divert attention away from vascular smooth muscle.

The role of NO is best investigated in the cerebral circulation where it plays an important role in the modulating response to acidosis, and where it is probably of extravascular origin (6). Several studies have shown increased

coronary blood flow during hypercapnia (18,19). Whether P_{CO_2} or pH exerts a direct effect on the smooth muscle of coronary vessels or whether the vascular response is mediated through other mechanisms is currently not known.

NO was proposed as a mediator of hypercapnia-evoked coronary vasodilation in the *in situ* dog heart (20) and findings in isolated aortic strips suggest that vasorelaxation in this artery in response to CO_2 may be partially mediated by NO (21). A number of publications have implicated NO in the vasodilatory response of the cerebral vasculature to hypercapnia (22), although more recent evidence suggests that the role of NO may be “permissive” rather than “causative” in this vascular bed (23).

Evidence from studying *in situ* dog hearts (20) came primarily from experiments in which the dilatory effects of hypercapnia on the coronary vasculature were attenuated (though not abolished) by prior exposure to the NO synthase (NOS) inhibitors L-NAME and L-NMMA. Furthermore, the coronary hypercapnic-induced vasodilation component that was sensitive to NOS inhibition was not secondary to increased blood flow, indicating that the release of NO was likely due to a direct effect of CO_2 (or H^+) on the vascular endothelium rather than to a shear stress-induced increase of coronary flow rate. No effect of the NOS inhibitors was observed on basal coronary flow, excluding the role of NO in the regulation of basal coronary vascular tone. This observation conflicts with *in vitro* findings in guinea pig Langendorff heart preparations, in which NOS inhibition reduced basal coronary vascular flow (21-23).

The role of NO in rat coronary flow regulation during acidosis was evaluated in isolated perfused rat Langendorff heart preparations exposed to brief periods of hypercapnic acidosis. Respiratory acidosis resulted in increased coronary flow, in conjunction with decreased contractile tension. Heart rate remained unaltered. The NOS inhibitor, L-NAME (100 μM), failed to attenuate the increases in coronary flow during hypercapnia or metabolic acidosis, even though it significantly reduced basal flow. These results suggest that, although NO contributes to the regulation of basal coronary vascular tone, it is not a mediator of the vasodilatory effects of hypercapnia and acidosis in this type of preparation (24).

Hypercapnia evokes responses that are both NO dependent and independent. One possible mechanism is hypercapnia-induced NO production via the L-arginine/NO pathway. However, NO donor restores the response to hypercapnia after prior attenuation with L-NAME. This would suggest that the production of NO may not be increased during hypercapnia and that the role of NO is merely permissive for the direct effect of pH change in vascular smooth muscle cells (23).

Respiratory alkalosis

Hyperoxia and alkalosis produce pulmonary vasodilation independent of endothelium-derived NO in newborn lambs; however, their mechanisms of action are unknown (25). NO is an important modulator of pulmonary vascular tone and produces potent pulmonary vasodilation during pulmonary hypertension. *In vitro* evidence suggests that NO may mediate the vasodilating effects of oxygen. To investigate whether NO synthesis mediates pulmonary vasodilation produced by hyperoxia, 100% oxygen normocapnic ventilation, arterial oxygen tension >450 torr (60 kPa) and alkalosis (hyperventilation with 21% oxygen, pH >7.55) were studied in 8 intact newborn lambs during similar degrees of pulmonary hypertension. It was reported that hyperoxia and alkalosis can produce pulmonary vasodilation independent of NO synthesis in the intact newborn lamb with pulmonary hypertensive disorders induced by U-46619 (a thromboxane A₂ mimic) or N-omega-nitro-L-arginine (an inhibitor of NO synthesis) (25).

It has been reported that alkalosis-induced vasodilation is mediated by NO in newborn piglet pulmonary artery and vein rings precontracted with U-46619. In contrast, prostacyclin or K^+ channel activation contributed to the response of *in situ* pulmonary vessels. A study that sought to identify factors contributing to the difference in reactivity between isolated and *in situ* pulmonary vessels found that NOS inhibition fully blocked alkalosis-induced relaxation of piglet artery and vein rings in isolated pulmonary preparations ventilated with a gas mixture containing 3% CO_2 (pH ~ 7.6) (26,27). In contrast, NOS inhibition alone had no effect on alkalosis-induced pulmonary vasodilation in isolated piglet lungs ventilated with gas mixture containing 0% CO_2 (pH ~ 7.6) (28). These data indicate that investigation of other factors, such as perivascular tissue (e.g., adventitia and parenchyma) and remote signaling pathways, should be carried out to reconcile this discrepancy in reactivity between isolated and *in situ* arteries (29).

Hypocapnic alkalosis-mediated relaxation is significantly blunted in piglet pulmonary venous rings without functional endothelium and in rings treated with NOS or guanylate cyclase inhibitor, suggesting that dilation is mediated by the NO-cGMP pathway (26) rather than by PGI_2 as reported by Hammerman et al. (30). In contrast to piglet pulmonary vessels, NO did not contribute to alkalosis-induced vasodilation in the rabbit (31) or rat (32) lung. PGI_2 synthesis, like NO synthesis, is enhanced by increased endothelial cell cytosolic Ca^{2+} (33). These findings reflect the interspecies differences in dominant endothelium-derived modulator synthesis.

Alkalosis causes a reduction in pulmonary vascular

resistance at normal and elevated tone conditions with the response limited primarily to the small arteries, which is not mediated by NO (32). The effects of alkalosis are abolished when vascular tone was increased with a low dose of KCl, suggesting that vascular response to pH may also involve changes in membrane potential.

Metabolic acidosis

For more than a century, it has been known that changes in pH are determinant of tone. Gaskell (34) demonstrated that acid solutions evoke vascular smooth muscle relaxation and more recent studies have shown that reduction of blood pH increases blood flow (35). This phenomenon, the so-called 'acidic-metabolic vasodilation', has been suggested to contribute to the regulation of local blood flow, mediating vasodilation that occurs during hypoxia or ischemia or during increased metabolic activity in order to fulfill the need for energy and oxygen.

A well-known phenomenon is that reduction of perivascular pH in acidemia decreases the responsiveness to vasoconstrictors and results in difficulty to maintain systemic blood pressure (36). Perivascular pH can affect many cellular processes (4), but the precise mechanisms of vascular hyporesponsiveness remain uncertain. Low pH has been shown to reduce Ca^{2+} influx (37), to inhibit myofilament contractility (38) and to alter receptors on the cell surface (39), accounting for the attenuation of vasoconstrictor responses.

Although these actions were observed with relatively large changes in pH, modest acidification (i.e., from pH 7.4 to 7.0) has also been demonstrated to cause substantial inhibition of vascular smooth muscle contractility (40), which has been in part associated with hyperpolarization (41) and an increase in intracellular Ca^{2+} sequestration. In addition to these direct actions of H^+ on smooth muscle, it has been demonstrated that endothelium-derived NO plays an important role in the hyporesponsiveness to vasoconstrictors elicited by acidemia (42).

Hattori et al. (43) examined the effects of modest acidification (pH_o 7.4 to 7.0) on the dilatory responses of isolated rat thoracic aorta and showed that modest acidification increases NO-mediated relaxation in rat aorta, probably due to an enhancement of cGMP-dependent pathway. In an acidic environment, NO seems to be more stable; additionally, relaxation was unrelated to $K^{(+)}$ channel mechanisms. NO is also implicated in cerebral arteriolar dilation because this was partially inhibited by L-NMMA and endothelial impairment. There are two possible explanations for endothelial NO-mediated vasodilation: 1) acidosis may activate NOS and 2) acidosis protects spontaneously released NO be-

cause NO is stable in acidic media (44).

Acidic vasodilation has mainly been suggested to be endothelium independent, but it is the enzymatically produced NO that has been suggested to be involved, making the origin of this NO unclear. In physiological concentration, nitrite evokes vasodilation, most likely through NO release. This NO release and parallel vasodilation is increased if the environmental pH is lowered to levels normally found in tissues after ischemia/hypoxia or increased metabolic activity. NO formation from nitrite is increased at low pH and acidic vasodilation by nitrite may involve cGMP. However, it is possible that some effects of NO may be related to other mechanisms such as direct interaction with ion channels. This suggests that non-enzymatically derived NO contributes to the "metabolic-acidic" local blood flow regulation in rat aorta (45).

In systemic vessels, acidosis causes vasodilation due to hyperpolarization of smooth muscles and it is possible that a similar potential effect may be present in pulmonary vessels. It has also been reported that acidosis inhibits calcium influx through "leak" channels in endothelial cells (46) and thus may inhibit NO synthesis. Activation of K_{ATP} channels and subsequent inhibition of Ca^{2+} influx via voltage dependent calcium channels is the mechanism by which a decrease in pH (with HCl) produces relaxation in denuded internal mammary artery (47).

Hypercapnic and eucapnic acidosis have different mechanisms between vascular beds. However, both lead to increased hydrogen ion concentration, and the direct and/or indirect effects are related to acidosis-induced dilatation via potassium and calcium channels in vascular smooth muscle. Smooth muscle K_{ATP} channels along with endothelium K_{ATP} channels contribute to acidosis-induced dilatation. The activation of potassium channels by low pH is responsible for cell membrane hyperpolarization. Other types of potassium channels (K_{Ca} , K_{ir} , K_v) seem to have varied roles in acidosis vasodilation depending on several factors, such as species and organ studied.

Most studies have associated acidosis with vascular dilatation attributed to cell hyperpolarization, decrease of intracellular calcium concentration, activation of potassium channels and NO. Although these may be the most common findings, some investigators have described acidic vasoconstriction in isolated aortas from rats. This indicates that vascular smooth muscle responses to acidic conditions are strain specific. Like strain specificity, the effects of acidosis are vascular-bed specific.

Metabolic alkalosis

Investigation of the effect of metabolic alkalosis on the

endothelium-dependent reactivity is rare. One paper mentioning possible effects of metabolic alkalosis relates the important role that NO plays in the transition from intrauterine to extrauterine life. If this transition fails, a condition called persistent pulmonary hypertension of the neonate may develop. The current treatment modalities for this disease include induction of alkalosis by hyperventilation or alkali infusion, inhaled NO and extracorporeal membrane oxygenation (48). During hyperventilation, lower P_{CO_2} values resulted in respiratory alkalosis, relaxed pulmonary vasculature and improved oxygenation. A similar increase in pulmonary blood flow can be obtained by intravenous infusion of alkaline solution that would increase pH by inducing metabolic alkalosis (49). There is evidence from animal studies that the elevated pH rather than the low P_{CO_2} is responsible for the resultant change in vascular resistance (50,51). Nagy et al. (49) examined the effect of pH on the activity and expression of endothelial NOS (eNOS) in cultured bovine aortic endothelial cells as a possible explanation for the pH-dependent drop in pulmonary vascular resistance. They did not observe eNOS expression after short- (4 h) and long-term (16 h) exposure to alkalosis (pH 7.1 to 7.6). Mizuno et al. (52) showed that increased extracellular pH activates eNOS via the influx of extracellular calcium and that the sodium/calcium exchanger regulates eNOS activity during alkalosis, collaborating for sustained activation of eNOS. Extracellular alkalosis is suggested to be involved in the stimulation of NO release via calcium influx. A close relationship exists between calcium uptake into endothelial cells and activation of the Na^+/Ca^{2+} exchanger, as well as a tight interaction of sodium/calcium exchanger with eNOS. Additionally intra-

cellular alkalization is also related to the activation of eNOS in association with Ca^{2+} influx, which is suppressed by inhibition of the Na^+/H^+ exchanger.

In metabolic alkalosis with compensatory hypercapnia, the subsequent constriction of the basilar artery due to lowered P_{CO_2} is prolonged, is independent of cerebral spinal fluid pH over a physiological range and is independent of NO and K_{ATP} channels (53).

Albuquerque and Leffler (54) reported that metabolic alkalosis (pH_o 7.7, P_{CO_2} 36 mmHg) in cultures of piglet cerebrovascular smooth muscle cells increased cytosolic calcium by about 80% as well as Ins(1,4,5)P₃. These results were also obtained for respiratory alkalosis. Thus, cerebral vasoconstriction probably involves the production of Ins(1,4,5)P₃ leading to cytosolic calcium elevation as a result of extracellular pH alteration.

Conclusion

NO, prostanoids and hyperpolarization may be involved in the mechanism responsible for pH-mediated vasodilation depending on vascular bed type. What remains to be determined is if their role is predominantly permissive for the direct effect of pH change on the smooth muscle cell. Another pattern that is emerging is that the altered pH_o can be associated with changes in pH_i and the latter may be involved in the vasodilator effects.

There is much information on acid base disorders in terms of the effects on vascular reactivity but it is quite divergent. Attention must be given to differences related to species, strain, type of vessel, vessel preparation, drugs used, and pre-tension given during experimental settings.

References

1. Smith GL, Austin C, Crichton C, Wray S. A review of the actions and control of intracellular pH in vascular smooth muscle. *Cardiovasc Res* 1998; 38: 316-331.
2. Ellis D, Thomas RC. Direct measurement of the intracellular pH of mammalian cardiac muscle. *J Physiol* 1976; 262: 755-771.
3. Aickin CC. Direct measurement of intracellular pH and buffering power in smooth muscle cells of guinea-pig vas deferens. *J Physiol* 1984; 349: 571-585.
4. Austin C, Wray S. Extracellular pH signals affect rat vascular tone by rapid transduction into intracellular pH changes. *J Physiol* 1993; 466: 1-8.
5. Franco-Cereceda A, Kallner G, Lundberg JM. Capsazepine-sensitive release of calcitonin gene-related peptide from C-fibre afferents in the guinea-pig heart by low pH and lactic acid. *Eur J Pharmacol* 1993; 238: 311-316.
6. Aalkjaer C, Poston L. Effects of pH on vascular tension: which are the important mechanisms? *J Vasc Res* 1996; 33: 347-359.
7. Ishizaka H, Kuo L. Acidosis-induced coronary arteriolar dilation is mediated by ATP-sensitive potassium channels in vascular smooth muscle. *Circ Res* 1996; 78: 50-57.
8. You JP, Wang Q, Zhang W, Jansen-Olesen I, Paulson OB, Lassen NA, et al. Hypercapnic vasodilatation in isolated rat basilar arteries is exerted via low pH and does not involve nitric oxide synthase stimulation or cyclic GMP production. *Acta Physiol Scand* 1994; 152: 391-397.
9. Zweier JL, Wang P, Samouilov A, Kuppusamy P. Enzyme-independent formation of nitric oxide in biological tissues. *Nat Med* 1995; 1: 804-809.
10. Giannella E, Mochmann HC, Levi R. Ischemic preconditioning prevents the impairment of hypoxic coronary vasodilata-

- tion caused by ischemia/reperfusion: role of adenosine A1/A3 and bradykinin B2 receptor activation. *Circ Res* 1997; 81: 415-422.
11. Aalkjaer C, Cragoe EJ Jr. Intracellular pH regulation in resting and contracting segments of rat mesenteric resistance vessels. *J Physiol* 1988; 402: 391-410.
 12. Aalkjaer C, Mulvany MJ. Effect of changes in intracellular pH on the contractility of rat resistance vessels. *Prog Biochem Pharmacol* 1988; 23: 150-158.
 13. Aickin CC. Regulation of intracellular pH in smooth muscle cells of the guinea-pig femoral artery. *J Physiol* 1994; 479 (Part 2): 331-340.
 14. Aalkjaer C, Peng HL. pH and smooth muscle. *Acta Physiol Scand* 1997; 161: 557-566.
 15. Caramelo C, Lopez-Farre A, Riesco A, Olivera A, Okada K, Cragoe EJ Jr, et al. Atrial natriuretic peptide and cGMP inhibit Na⁺/H⁺ antiporter in vascular smooth muscle cells in culture. *Kidney Int* 1994; 45: 66-75.
 16. Korbmacher C, Helbig H, Stahl F, Wiederholt M. Evidence for Na/H exchange and Cl/HCO₃ exchange in A10 vascular smooth muscle cells. *Pflugers Arch* 1988; 412: 29-36.
 17. Wakabayashi I, Poteser M, Groschner K. Intracellular pH as a determinant of vascular smooth muscle function. *J Vasc Res* 2006; 43: 238-250.
 18. Clancy RL, Gonzalez NC. Effect of respiratory and metabolic acidosis on coronary vascular resistance (38528). *Proc Soc Exp Biol Med* 1975; 148: 307-311.
 19. Ely SW, Sawyer DC, Scott JB. Local vasoactivity of oxygen and carbon dioxide in the right coronary circulation of the dog and pig. *J Physiol* 1982; 332: 427-439.
 20. Gurevicius J, Salem MR, Metwally AA, Silver JM, Crystal GJ. Contribution of nitric oxide to coronary vasodilation during hypercapnic acidosis. *Am J Physiol* 1995; 268: H39-H47.
 21. Fukuda S, Matsumoto M, Nishimura N, Fujiwara N, Shimoji K, Takeshita H, et al. Endothelial modulation of norepinephrine-induced constriction of rat aorta at normal and high CO₂ tensions. *Am J Physiol* 1990; 258: H1049-H1054.
 22. Kelm M, Schrader J. Control of coronary vascular tone by nitric oxide. *Circ Res* 1990; 66: 1561-1575.
 23. Kostic MM, Petronijevic MR, Jakovljevic VL. Role of nitric oxide (NO) in the regulation of coronary circulation. *Physiol Res* 1996; 45: 273-278.
 24. Song D, O'Regan MH, Phillis JW. Role of nitric oxide in rat coronary flow regulation during respiratory and metabolic acidosis. *Gen Pharmacol* 1999; 32: 571-575.
 25. Fineman JR, Wong J, Soifer SJ. Hyperoxia and alkalosis produce pulmonary vasodilation independent of endothelium-derived nitric oxide in newborn lambs. *Pediatr Res* 1993; 33: 341-346.
 26. Halla TR, Madden JA, Gordon JB. Mediators of alkalosis-induced relaxation of piglet pulmonary veins. *Am J Physiol Lung Cell Mol Physiol* 2000; 278: L968-L973.
 27. Gordon JB, Halla TR, Fike CD, Madden JA. Mediators of alkalosis-induced relaxation in pulmonary arteries from normoxic and chronically hypoxic piglets. *Am J Physiol* 1999; 276: L155-L163.
 28. Vander Heyden MA, Halla TR, Madden JA, Gordon JB. Multiple Ca²⁺-dependent modulators mediate alkalosis-induced vasodilation in newborn piglet lungs. *Am J Physiol Lung Cell Mol Physiol* 2001; 280: L519-L526.
 29. Gordon JB, VanderHeyden MA, Halla TR, Cortez EP, Hernandez G, Haworth ST, et al. What leads to different mediators of alkalosis-induced vasodilation in isolated and *in situ* pulmonary vessels? *Am J Physiol Lung Cell Mol Physiol* 2003; 284: L799-L807.
 30. Hammerman C, Aramburo MJ. Effects of hyperventilation on prostacyclin formation and on pulmonary vasodilation after group B beta-hemolytic streptococci-induced pulmonary hypertension. *Pediatr Res* 1991; 29: 282-287.
 31. Yamaguchi K, Takasugi T, Fujita H, Mori M, Oyamada Y, Suzuki K, et al. Endothelial modulation of pH-dependent pressor response in isolated perfused rabbit lungs. *Am J Physiol* 1996; 270: H252-H258.
 32. Gao Y, Tassiopoulos AK, McGraw DJ, Hauser MC, Camporesi EM, Hakim TS. Segmental pulmonary vascular responses to changes in pH in rat lungs: role of nitric oxide. *Acta Anaesthesiol Scand* 1999; 43: 64-70.
 33. Ritter JM, Frazer CE, Taylor GW. pH-dependent stimulation by Ca²⁺ of prostacyclin synthesis in rat aortic rings: effects of drugs and inorganic ions. *Br J Pharmacol* 1987; 91: 439-446.
 34. Gaskell WH. On the tonicity of the heart and blood vessels. *J Physiol* 1880; 3: 48-92.
 35. Zsoter T, Banderman L, Chappel CI. The effect of "local" pH changes on blood flow in the dog. *Am Heart J* 1961; 61: 777-782.
 36. Dusting GJ, Rand MJ. Interactions between the hydrogen ion concentration and vasoconstrictor responses to catecholamines and sympathetic nerve stimulation. *Clin Exp Pharmacol Physiol* 1975; Suppl 2: 43-48.
 37. Kohlhardt M, Haap K, Figulla HR. Influence of low extracellular pH upon the Ca inward current and isometric contractile force in mammalian ventricular myocardium. *Pflugers Arch* 1976; 366: 31-38.
 38. Fabiato A, Fabiato F. Effects of pH on the myofilaments and the sarcoplasmic reticulum of skinned cells from cardiac and skeletal muscles. *J Physiol* 1978; 276: 233-255.
 39. Flavahan NA, McGrath JC. Alpha 1-adrenoceptors can mediate chronotropic responses in the rat heart. *Br J Pharmacol* 1981; 73: 586-588.
 40. Rooke TW, Sparks HV Jr. Effect of metabolic versus respiratory acid-base changes on isolated coronary artery and saphenous vein. *Experientia* 1981; 37: 982-983.
 41. Bonnet P, Rusch NJ, Harder DR. Characterization of an outward K⁺ current in freshly dispersed cerebral arterial muscle cells. *Pflugers Arch* 1991; 418: 292-296.
 42. Fukuda S, Morioka M, Tanaka T, Taga K, Shimoji K. Endothelium dependence of effects of high PCO₂ on agonist-induced contractility of rat aorta. *Am J Physiol* 1993; 264: H512-H519.
 43. Hattori K, Tsuchida S, Tsukahara H, Mayumi M, Tanaka T, Zhang L, et al. Augmentation of NO-mediated vasodilation in metabolic acidosis. *Life Sci* 2002; 71: 1439-1447.
 44. Horiuchi T, Dietrich HH, Hongo K, Goto T, Dacey RG Jr. Role of endothelial nitric oxide and smooth muscle potassium channels in cerebral arteriolar dilation in response to acidosis. *Stroke* 2002; 33: 844-849.
 45. Modin A, Bjorne H, Herulf M, Alving K, Weitzberg E, Lundberg JO. Nitrite-derived nitric oxide: a possible mediator of 'acidic-metabolic' vasodilation. *Acta Physiol Scand* 2001; 171: 9-16.

46. Demirel E, Laskey RE, Purkerson S, van Breemen C. The passive calcium leak in cultured porcine aortic endothelial cells. *Biochem Biophys Res Commun* 1993; 191: 1197-1203.
47. Rohra DK, Sharif HM, Zubairi HS, Sarfraz K, Ghayur MN, Gilani AH. Acidosis-induced relaxation of human internal mammary artery is due to activation of ATP-sensitive potassium channels. *Eur J Pharmacol* 2005; 514: 175-181.
48. Kinsella JP, Abman SH. Recent developments in the pathophysiology and treatment of persistent pulmonary hypertension of the newborn. *J Pediatr* 1995; 126: 853-864.
49. Nagy S, Harris MB, Ju H, Bhatia J, Venema RC. pH and nitric oxide synthase activity and expression in bovine aortic endothelial cells. *Acta Paediatr* 2006; 95: 814-817.
50. Lyrene RK, Welch KA, Godoy G, Philips JB III. Alkalosis attenuates hypoxic pulmonary vasoconstriction in neonatal lambs. *Pediatr Res* 1985; 19: 1268-1271.
51. Schreiber MD, Heymann MA, Soifer SJ. Increased arterial pH, not decreased PaCO₂, attenuates hypoxia-induced pulmonary vasoconstriction in newborn lambs. *Pediatr Res* 1986; 20: 113-117.
52. Mizuno S, Demura Y, Ameshima S, Okamura S, Miyamori I, Ishizaki T. Alkalosis stimulates endothelial nitric oxide synthase in cultured human pulmonary arterial endothelial cells. *Am J Physiol Lung Cell Mol Physiol* 2002; 283: L113-L119.
53. Yoon SH, Zuccarello M, Rapoport RM. Reversal of hypercapnia induces KATP channel and NO-independent constriction of basilar artery in rabbits with acute metabolic alkalosis. *Gen Pharmacol* 2000; 35: 325-332.
54. Albuquerque ML, Leffler CW. pHo, pH_i, and PCO₂ in stimulation of IP₃ and [Ca²⁺]_i in piglet cerebrovascular smooth muscle. *Proc Soc Exp Biol Med* 1998; 219: 226-234.