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MINIREVIEW

Changes in Smooth Muscle Cell pH during Hypoxic Pulmonary Vasoconstriction: A Possible Role for Ion Transporters

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Summary

Hypoxic pulmonary vasoconstriction (HPV) occurs in smooth muscle cells (SMC) from small pulmonary arteries (SPA) and is accompanied by increases in free cytoplasmic calcium ($[Ca^{2+}]_i$) and cytoplasmic pH (pH_i). SMC from large pulmonary arteries (LPA) relax during hypoxia, and $[Ca^{2+}]_i$ and pH_i decrease. Increases in pH_i and $[Ca^{2+}]_i$ in cat SPA SMC during hypoxia and the augmentation of hypoxic pulmonary vasoconstriction by alkalosis seen in isolated arteries and lungs suggest that cellular mechanisms, which regulate inward and outward movement of Ca^{2+} and H^+, may participate in the generation of HPV. SMC transport systems that regulate pH_i include the Na^+-H^+ transporter which regulates intracellular Na^+ and H^+ and aids in recovery from acid loads, and the Na^+-dependent and Na^+-independent Cl^-/HCO_3^- transporters which regulate intracellular chloride. The Na^+-dependent Cl^-/HCO_3^- transporter also aids in recovery from acidosis in the presence of CO_2 and HCO_3^-. The Na^+-independent Cl^-/HCO_3^- transporter aids in recovery from cellular alkalosis. The Na^+-H^+ transporter was present in SMC from SPA and LPA of the cat, but it seemed to have little if any role in regulating pH_i in the presence of CO_2 and HCO_3^-. Inhibiting the Cl^-/HCO_3^- transporters reversed the normal direction of pH_i change during hypoxia, suggesting a role for these transporters in the hypoxic response. Future studies to determine the interaction between pH_i, $[Ca^{2+}]_i$ and HPV should ascertain whether pH_i and $[Ca^{2+}]_i$ changes are linked and how they may interact to promote or inhibit SMC contraction.

Key words

Cat • Na^+/H^+ exchange • Na^+-dependent Cl^-/HCO_3^- exchange • Na^+-independent Cl^-/HCO_3^- exchange • pH • H^+ ion

Hypoxic pulmonary vasoconstriction

In the normal lung, hypoxic pulmonary vasoconstriction diverts blood flow from poorly to better ventilated areas and seems to be primarily a function of the small (200-600 μm diameter) resistance arteries (SPA; Madden *et al.* 1985). Although endothelial factors may modulate the magnitude of the hypoxic contraction, the contractile process appears to be localized in the smooth muscle cells (Madden *et al.* 1992). The

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contraction to hypoxia is accompanied by the increases in both free cytoplasmic calcium concentration ($[Ca^{2+}]_i$; Subramanian 1993) and cytoplasmic pH (pH_i; Vadula *et al.* 1992a,b, Ray *et al.* 1994) In contrast to the behavior of SPA smooth muscle cells, cells from larger (>800 μm diameter) pulmonary arteries (LPA) relax in response to hypoxia and their $[Ca^{2+}]_i$ and pH_i decrease (Madden *et al.* 1992, Vadula *et al.* 1992a,b).

Changes in $[Ca^{2+}]_i$ are primary events in the contractile and relaxant processes of vascular smooth muscle, whereas changes in pH_i can alter Ca^{2+}-calmodulin binding and thus, the magnitude of the contractile response for a given $[Ca^{2+}]_i$ concentration. Changes in pH_i can alter Ca^{2+} channel conductance in cellular and organelle membranes and affect Ca^{2+}-dependent steps in the contractile process (Wray 1988, Aalkjaer 1990). These pH_i sensitive Ca^{2+}-dependent contractile steps include: 1) myosin ATPase activity, 2) contractile unit Ca^{2+} sensitivity, 3) Ca^{2+}-calmodulin binding, and 4) Ca^{2+} binding-site sensitivity (Wray 1988). When pH_i decreases, the Ca^{2+} requirement for contraction increases and the Ca^{2+} sensitivity of contractile proteins is reduced. On the other hand, when pH_i increases, the vascular smooth muscle cell depolarizes, voltage-dependent Ca^{2+} channels open, and the Ca^{2+} sensitivity of arterial muscle contractile element increases. The increased $[Ca^{2+}]_i$ binds to calmodulin and activates Ca^{2+}-calmodulin-dependent enzymes. Whether hypoxic pulmonary vasoconstriction requires an effect of pH_i on $[Ca^{2+}]_i$ or *vice versa* is not known.

Effects of increasing pH on hypoxic pulmonary vasoconstriction

For many years it has been accepted that extracellular alkalosis attenuates the increased pulmonary vascular resistance produced by hypoxia (Sylvester *et al.* 1986). However, recent evidence suggests that pulmonary vascular responses to alkalosis may reflect a balance between enhanced modulation of vasomotor tone and increased vascular smooth muscle contractility due to an increase in pH_i. For example, in isolated arteries and lungs even though relaxation occurs when extracellular pH (pH_O) is increased, constrictor responses to hypoxia and other agonists are also augmented (Gordon *et al.* 1993, Raffestin *et al.* 1987, Krampetz and Rhoades 1991). For example, isolated small pulmonary arteries exposed to hypocarbia to increase pH_O (7.6), showed a transient decrease in tone (Gordon *et al.* 1993). However, after 30 min of alkalosis, tone returned to the prealkalosis level. When the vessels were then exposed to hypoxia under alkalotic conditions, the contractile response was threefold greater ($P < 0.05$) than at pH_O 7.4. A similar protocol in isolated lamb lungs also revealed that even though the pulmonary artery pressure decreased during alkalosis, hypoxic reactivity after alkalosis was enhanced (Gordon *et al.* 1993) Since hypocarbia would also tend to increase pH_i, this result is consistent with the notion that pH_i modulates the response to hypoxia.

Intracellular alkalosis might not necessarily result in an immediate contractile response of the smooth muscle cell but it might increase the filling of intracellular Ca^{2+} stores through effects on the mechanisms that regulate $[Ca^{2+}]_i$, among them, ion transporters. Intracellular alkalosis maintained over several cell cycles has also been shown to cause profound phenotypic and genotypic alterations (Oberleithner and Schwab 1994). If prolonged hypoxia results in a sustained increase in pH_i, this might lead to changes in the vascular smooth muscle cell that would be conducive to the development of pulmonary hypertension. Actively proliferating cells have a more alkaline pH_i than cells in a growth-arrested state (Batlle *et al.* 1991) and it has been shown that the activity of the Na^+-H^+ transporter is altered in growing cells (Batlle *et al.* 1991).

The concomitant increases in pH_i and $[Ca^{2+}]_i$ in cat SPA smooth muscle cells during hypoxia and the augmentation of hypoxic pulmonary vasoconstriction by alkalosis in isolated arteries and lungs suggest that cellular mechanisms that regulate the movement of Ca^{2+} and H^+ into and out of the smooth muscle cell may play a significant role in the generation of the hypoxic response. While the interaction and regulation of pH_i and $[Ca^{2+}]_i$ in the pulmonary vasculature are undoubtedly multifaceted processes, one important way by which they occur is through the activities of the smooth muscle cell ion transporters.

Ion transporters

Smooth muscle cells contain several transport systems that regulate pH_i. The three primary transporters that acidify and alkalinize the cell are shown in Figure 1. Different cell types can vary in their expression of a particular transporter, and within the same cell type one transporter may predominate under steady-state conditions and another during agonist stimulation. Vasoactive agonists can also affect the activity and the

direction of a transporter within the smooth muscle cell (Aalkjaer 1990, Wray 1988, O'Donnell and Owen 1994). For the cell to respond appropriately, the operation of the ion transporters must be coordinated. For example, agonist stimulation of an transporter may increase the cycling of an ion between the cell and its environment. This, in turn, may stimulate other transporters to restore ionic homeostasis (O'Donnell and Owen 1994).

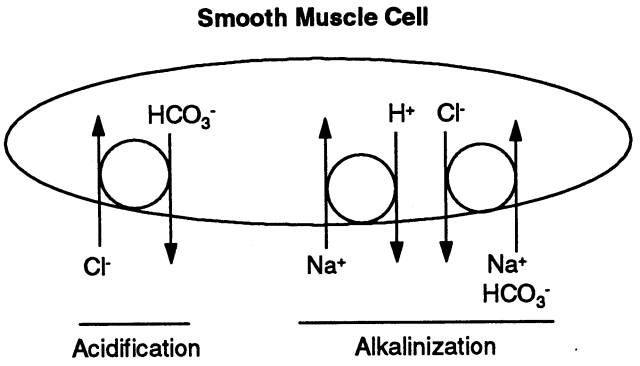

Fig 1. *Major ion transporters that regulate pH in a smooth muscle cell.*

Na^+-H^+ transporter

The Na^+-H^+ transporter regulates Na^+_i, pH_i and cell volume, and it plays an essential role in agonist-mediated cell proliferation (Takewaki *et al.* 1995). It also appears to play a dominant role in the recovery from acid loads as may occur during anaerobic metabolism or in response to a stimulus. When the transporter is inhibited by reducing extracellular Na^+ to low levels or by using specific Na^+-H^+ exchange blockers such as amiloride analogs, cellular H^+ concentration increases.

The activity of the Na^+-H^+ transporter has been demonstrated in many types of vascular smooth muscle (O'Donnell and Owen 1994). Within the pulmonary circulation there has been indirect evidence of its activity in ferret pulmonary arteries (Farrukh *et al.* 1996) and direct evidence in smooth muscle cells from guinea pig main pulmonary artery (Quinn *et al.* 1991). In these cells it played an active role in regulating pH_i, even in the presence of HCO_3^--containing buffers. We have found that the Na^+-H^+ transporter also appears to be present in both SPA and LPA smooth muscle cells from the cat but it seems to play little if any role in regulating pH_i in the presence of physiologic P_{CO2} and HCO_3^- (Madden *et al.* 1999).

Na^+-dependent and Na^+-independent Cl^-/HCO_3^- transporters

Both the Na^+-dependent and the Na^+-independent Cl^-/HCO_3^- transporters regulate intracellular Cl, and they may be major pathways for Cl^- movement across the cell membrane (O'Donnell and Owen 1994). The Na^+-dependent Cl^-/HCO_3^- transporter plays a prominent role in recovery from an acidosis, and in the presence of CO_2 and HCO_3^- it may have a greater role than the Na^+-H^+ transporter in maintaining normal pH_i in unstimulated cells (Kahn *et al.* 1991). The Cl^-/HCO_3^- transporter has been demonstrated in guinea pig main pulmonary artery (Quinn *et al.* 1991) and in cat SPA and LPA cells (Madden *et al.* 1999). We found that the characteristic pH_i responses to hypoxia by SPA and LPA smooth muscle cells mentioned above are reversed by inhibiting the Cl^-/HCO_3^- transporters with the disulfonic stilbene, DIDS (Madden *et al.* 1999). These findings suggest that the Cl^-/HCO_3^- transporters play a major role in the pH_i changes that occur during hypoxia .

Two other transporters that may also help to regulate $[Ca^{2+}]_i$ and pH_i are less well studied, particularly within the pulmonary circulation. These are the Ca^{2+}-Mg^{2+} ATPase and the H^+-K^+ ATPase

Ca^{2+}-stimulated-Mg^{2+}-dependent ATPase

Ca^{2+}-Mg^{2+} ATPases are membrane-bound proteins found in both the plasma membrane and the sarcoplasmic reticulum. One of the primary functions of the Ca^{2+}-Mg^{2+} ATPase is to exchange one H^+ produced during ATP hydrolysis for one Ca^{2+} (Carafoli 1991). Because of this function, the Ca^{2+}-Mg^{2+} ATPase is sometimes called the Ca^{2+}-H^+ transporter. In skeletal muscle, pH changes alter the activity of the Ca^{2+}-Mg^{2+} ATPases (Wolosker and De Meis 1994) . In smooth muscle, the Ca^{2+}-Mg^{2+} ATPases in conjunction with the Na^+-Ca^{2+} transporter help to regulate the Ca^{2+} content of the cytosol and thus modulate smooth muscle cell tone. The activity of the plasma membrane Ca^{2+}-Mg^{2+} ATPase is stimulated by calmodulin, cGMP-dependent protein kinase, and perhaps IP_3 (O'Donnell and Owen 1994).

The sarcoplasmic reticulum Ca^{2+}-Mg^{2+} ATPase has been found to be highly active in bovine main pulmonary artery (Kwan 1982) where it pumps Ca^{2+} from the cytoplasm into the sarcoplasmic reticulum to maintain

or restore the normally low levels of $[Ca^{2+}]_i$. Oxidative damage can inhibit the sarcoplasmic reticulum Ca^{2+}-Mg^{2+} ATPase but whether hypoxia also inhibits it is not known. However, inhibiting Ca^{2+}-Mg^{2+} ATPase activity did increase the hypoxic response of isolated ferret lungs (Farrukh and Michael 1992).

H^+-K^+ ATPase

Evidence for this transporter comes from physiological studies as well as molecular studies showing mRNA for the gastric-type isoform of the H^+-K^+ ATPase (Marrelli *et al.* 1997). However, a role for this transporter in the hypoxic response of smooth muscle cells, particularly in pulmonary vessels, has not been examined.

A role for ion transporters in the hypoxic response of pulmonary artery smooth muscle cells?

Under normoxic conditions, smooth muscle cells from cat LPA have a generally higher resting pH_i than cells from SPA (Madden *et al.* 1999). During hypoxia, however, the pH_i from the LPA decreases whereas the pH_i in the SPA increases. Changing the resting pH_i of the one cell type to that of the other does not reverse this trend. It thus appears that the pH_i changes during hypoxia are a response to hypoxia rather than a function of the baseline pH_i.

The activities of the ion transporters were evaluated in the cat SPA and LPA smooth muscle cells by conventional physiological maneuvers rather than by determination of specific molecular identities. Using the NH_4Cl washout technique in CO_2 and HCO_3^--free solutions, we demonstrated that smooth muscle cells from both SPA and LPA possessed the Na^+-H^+ transporter. However, in CO_2 and HCO_3^--containing solutions, the Na^+-H^+ transporter appeared to contribute little to steady-state pH_i maintenance or to recovery from an acid load. Under hypoxic conditions and in the presence of CO_2 and HCO_3^-, the increase in pH_i in SPA cells was not due to reverse activation of Na^+-H^+ exchange since the pH_i still increased in dimethylamiloride (DMA) – treated SPA cells.

The Na^+-independent Cl^-/HCO_3^- transporter also appeared to play a role in determining steady-state pH_i in both types of pulmonary artery smooth muscle cells. When Cl^- was removed from the CO_2 and HCO_3^- solution bathing the cells, the pH_i increased in both SPA and LPA. This response indicated intracellular alkalinization due to Cl^- efflux and HCO_3^- influx, or to an absence of extracellular Cl^- to exchange for intracellular HCO_3^-. When extracellular Cl^- was restored, the pH_i returned to baseline levels. However, in the presence of DIDS, pH_i remained above baseline in both cell types. If Cl^- was restored but Na^+ was removed, pH_i declined to a level significantly below baseline indicating that neither the Na^+-dependent Cl^-/HCO_3^- nor the Na^+-H^+ transporter prevented the acidification produced by the activity of the Na^+-independent Cl^-/HCO_3^- transporter. When smooth muscle cells of both types were bathed in HCO_3^--PSS + DIDS, the pH_i responses to hypoxia were reversed. The pH_i of the SPA cells decreased rather than increased during hypoxia whereas the pH_i of the LPA cells rose rather than decreased.

On the basis of the findings in the cat pulmonary artery smooth muscle cells one might speculate that the pH_i of cells under normoxic and hypoxic conditions is determined by the relative balances of the alkalinizing Na^+-dependent Cl^-/HCO_3^- transporter and the acidifying Na^+-independent Cl^-/HCO_3^- transporter. During normoxia, the relative abundance or the affinity for substrate of these transporters might be such that activity of the Na^+-independent Cl^-/HCO_3^- transporter would contribute more towards maintaining pH_i in SPA cells than would the activity of the Na^+-independent Cl^-/HCO_3^- transporter. In the LPA cells, however, the balance would favor the Na^+-dependent Cl^-/HCO_3^- transporter. Such a scenario of different relative activities between these transporters would keep the pH_i in SPA cells at a more acidic level than the LPA cells. Exposing SPA cells to hypoxia might either directly activate the Na^+-dependent Cl^-/HCO_3^- transporter or mobilize it from an intracellular compartment to the cell membrane so that pH_i would increase. In the LPA cells, hypoxia would similarly activate or mobilize the Na^+-independent Cl^-/HCO_3^- transporter so that pH_i would decrease. The initial change in pH_i would continue until enough stimulation of a countervailing transporter is induced by the new level of pH_i or cell HCO_3^- so that a new steady-state is achieved.

Future directions

Future studies to determine the interaction between pH_i, $[Ca^{2+}]_i$, and hypoxic pulmonary vasoconstriction would require ascertaining whether changes in pH_i and $[Ca^{2+}]_i$ are linked to one another and

how they interact to promote or inhibit contraction of smooth muscle. In addition, studies should be done to determine whether the responses to hypoxia of LPA and SPA cells are based on differences in gene products expressed in the two smooth muscle cell types. Such studies would not only elucidate the roles of pH_i and $[Ca^{2+}]_i$ in hypoxic pulmonary vasoconstriction but they would also determine some of the signaling molecules that sense hypoxia and communicate this stimulus to the acid-base transporters and, possibly, regulatory gene products involved in this phenomenon.

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