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MINIREVIEW

Possible Mechanisms of Oxygen Sensing in the Pulmonary Circulation

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Summary

Oxygen tension is known to control the pulmonary vascular tone. We reviewed three hypotheses that try to explain the mechanism whereby hypoxia is sensed in the lung tissue. The first hypothesis concerns the role of the oxygen binding hemoprotein cytochrome P-450. Studies using various inhibitors and activators of cytochrome P-450 show that this enzyme affects pulmonary vascular tone. The data are, however, contradictory. The second hypothesis postulates that hypoxia reduces the synthesis of vasodilator oxygen radicals in the lung. This hypothesis is quite well supported by experimental data. The third hypothesis, similarly widely documented, states that slowing of the respiratory chain and altered cellular energetics is crucial for sensing of hypoxia. In this case, however, it is not exactly clear how changes in cellular energetics are connected with vascular tone. The possibility exists that changes in both the cytochrome P-450 activity and in the rate of electrons flow in the respiratory chain may alter the amount of oxygen radicals in the cells and, similarly as in the "oxygen radicals" hypothesis, govern calcium channels through the control of the redox status of these channels.

Key words
Hypoxic pulmonary vasoconstriction – Pulmonary circulation – Hypoxia – Oxygen radicals

Pulmonary vasoconstriction in response to alveolar hypoxia helps to prevent arterial hypoxemia by matching blood flow through individual acini to their ventilation (Grant 1987). Hypoxic pulmonary vasoconstriction is a chain of events including oxygen sensor, transducer of the information about hypoxia from sensor to effector, and effector (contractile apparatus of smooth muscle of pulmonary arterioles). The present review deals with the current hypotheses about the nature of the first step, the sensor of hypoxia.

The rate of a biochemical reaction can be regulated either allostericaly or by the availability of substrates. Hence, two types of enzymatic reactions can, hypothetically, serve for oxygen sensing. The first type is a reaction catalyzed by

an enzyme that is allostericaly regulated by an oxygen molecule. This interesting hypothesis has not been experimentally investigated so far. The second possibility is a reaction in which oxygen plays a role of the substrate. The rate of such a reaction will be lowered by hypoxia. The number of reactions utilizing oxygen (catalyzed by enzymes called oxygenases) is high. Three of them, the activity of microsomal cytochrome P-450, the production of oxygen radicals, and the mitochondrial oxidative phosphorylation, have received attention in terms of hypoxic pulmonary vasoconstriction.

Cytochrome P-450

Cytochrome P-450 is an oxygen binding hemoprotein present in the lung. Sylvester and McGowan (1978) suggested that alveolar hypoxia may be linked to pulmonary vasoconstriction by the changes in cytochrome P-450 saturation with oxygen. Hypoxic pulmonary vasoconstriction was found to be inhibited by cytochrome P-450 blockers carbon monoxide (Duke and Killick 1952, Sylvester and McGowan 1978, Miller and Hales 1979) and metyrapone (Sylvester and McGowan 1978, Miller and Hales 1979). In other studies, however, hypoxic pulmonary vasoconstriction was not depressed by CO (Marshall *et al.* 1988), metyrapone (Custer *et al.* 1985), or another cytochrome P-450 blocker 1-aminobenzotriazole (Voelkel *et al.* 1989). On the other hand, hypoxic pulmonary vasoconstriction was inhibited by cytochrome P-450 stimulators B-naphtaflavone and 3-methylcholanthrene (Voelkel *et al.* 1989). Even though the situation may be complicated by nonspecific side effects of the drugs used (e.g. nonspecific depression of reactivity in the study of Sylvester and McGowan but not in that of Custer's group), it is likely that cytochrome P-450 is able to contribute to the regulation of the pulmonary vascular tone. The mechanism of this involvement (metabolism of vasoactive substances?), however, remains unclear. Furthermore, its role during hypoxia is questionable since the cytochrome P-450 activity and the pulmonary vascular tone are altered by different degrees of hypoxia (Knoblauch *et al.* 1981).

Oxygen radicals

Weir and Will (1982) have suggested that a vasodilator action of oxygen radicals produced in the course of normal biochemical pathways is responsible for the low tone of pulmonary vessels in normoxia. If so, hypoxic pulmonary vasoconstriction will be a result of the reduced amount of radicals produced in hypoxia. This hypothesis is supported by numerous data.

Hypoxia reduces the total lung radicals content, and this occurs immediately before the vascular tone starts to rise (Archer *et al.* 1989b). A quick return of the amount of radicals to a basal level is caused by reoxygenation and is followed by vasodilation (Archer *et al.* 1989b).

Exogenous oxidants including free radicals (H_2O_2, superoxide, t-butyl hydroperoxide, 2-butanone peroxide, diamide) blunt, some of them specifically, the pulmonary vascular reactivity to acute hypoxia (Weir and Will 1982, Weir *et al.*

1983, Burghuber *et al.* 1984, Archer *et al.* 1989c,e). The inhibition of glutathione reductase has similar effect (Peters-Golden *et al.* 1989). This enzyme supplies the cell with its main antioxidant, reduced glutathione (Flenley 1987). The blockade of glutathione reductase should, therefore, enhance the amount of endogenous radicals. By contrast, radicals scavengers (catalase and superoxide dismutase) enhance basal tone as well as reactivity of the pulmonary vessels (Archer *et al.* 1989e). This applies only for enzymes administered in liposomes. It suggests that intracellular rather than extracellular radicals are important.

There are two views on the way in which oxygen radicals might affect the pulmonary vascular tone. The first one is based on the fact that the function of voltage-dependent calcium channels is influenced by their redox status (reviewed by Archer and Weir 1989). A drop in oxygen radicals content during hypoxia might increase the degree of activation of voltage-dependent channels. The resulting rise in intracellular calcium concentration would activate contractile proteins of a vascular smooth muscle (Karaki and Weiss 1988).

The second view postulates a role of cyclic guanosine monophosphate (GMP). A transient form of catalase during H_2O_2 degradation, compound I, stimulates a cytoplasm-bound form of guanylate cyclase (Burke and Wolin 1987). Cyclic GMP causes relaxation and hyporeactivity of the pulmonary arterial smooth muscle (Murad *et al.* 1987). Sin 1, a direct stimulator of guanylate cyclase, causes dose-dependent vasodilation in the isolated rat lungs (Marshall and Marshall 1990). Inhibition of catalase by aminotriazole or the blockade of guanylate cyclase by methylene blue augments the tone of pulmonary arterioles (Burke-Wolin and Wolin 1989, Hyman *et al.* 1989, Marshall and Marshall 1990). There are, however, controversial data about the effect of guanylate cyclase inhibition on the pulmonary reactivity to vasoconstrictor stimuli. Blunting (Burke-Wolin and Wolin 1989, Marshall and Marshall 1990) as well as potentiation (Yamaguchi *et al.* 1987, Rodman *et al.* 1988, Mazmanian *et al.* 1989) were noted. Moreover, changes in the pulmonary vascular tone and cyclic GMP content are not always reciprocal. Serotonin, a pulmonary vasoconstrictor (Hofman and Ehrhart 1987), enhances the amount of cyclic GMP in the lungs (Burke and Wolin 1987). Exogenous catalase enhances, not attenuates, the pulmonary vascular tone (Archer *et al.* 1989e). The role of cyclic GMP in the regulation of the pulmonary vascular smooth muscle thus remains controversial.

Besides this disagreement about the mechanism of the vasodilative action of radicals, there are several experimental findings that seem not to be consistent with the oxygen radicals hypothesis. The administration of exogenous radicals renders the pulmonary vessels hyporeactive to subsequent vasoconstrictor stimuli (Archer *et al.* 1989e, Rhoades *et al.* 1990). The exogenous oxygen radicals were found to cause vascular smooth muscle contraction in isolated rabbit lungs and in *in vitro* rat pulmonary artery rings (Tate *et al.* 1982, Seeger *et al.* 1986, Rhoades *et al.* 1990). This may be a consequence of toxic action of higher doses of radicals. Some antioxidants may blunt rather than potentiate hypoxic pulmonary vasoconstriction (Haynes *et al.* 1987, Chang and Voelkel 1986). An important problem of the oxygen radicals hypothesis is that alteration of the radicals concentration must be somewhat localized, since overall changes of the redox status in the entire cell would significantly perturb many enzymatic reactions.

Oxidative phosphorylation

In the course of oxidative phosphorylation, the electrons are transferred by a chain of transporters in the inner mitochondrial membrane (the last one is cytochrome aa_3) from Krebs cycle to molecular oxygen. Redox energy dissipated by the respiratory chain is used for generation of electrochemical potential difference of hydrogen ions across the inner mitochondrial membrane. This proton-motive gradient drives F_0F_1-ATPase, which phosphorylates ADP to ATP. The "energy" hypothesis of lung oxygen sensing suggests that the key step of the sensor mechanism is slowdown of cytochrome aa_3 by the lack of its substrate – oxygen.

If this hypothesis is valid, the inhibition of the respiratory chain by means other than hypoxia should also cause pulmonary vasoconstriction. In fact, pulmonary vasoconstriction may be elicited by rotenone, antimycin A, azide, and cyanide (Rounds and McMurtry 1981, Lloyd 1964, 1965). These substances inhibit various steps of the electron transport in mitochondria. Their vasoconstrictive action in the lungs is similar to hypoxic pulmonary vasoconstriction in sensitivity to Ca^{2+} channels inhibitors (Rounds and McMurtry 1981) and to cooling, prolonged perfusion or absence of blood cells in perfusate (Lloyd 1964, 1965). The pressor response to metabolic inhibitors, however, is transient and causes subsequent vascular paresis. Selective inhibition of cytochrome aa_3 by carbon monoxide significantly potentiates vasopressor response to the slight degree of hypoxia in isolated rat lungs (Marshall *et al.* 1988).

The respiratory chain may be blocked not only by pharmacologic inhibitors of its transporters but also by attenuation of the electron delivery to the chain, i.e. by slowing of the Krebs cycle. The Krebs cycle inhibitors fluoroacetate or malonate elevate pulmonary arterial pressure in dogs (Liang 1977) and potentiate hypoxic pulmonary vasoconstriction in the isolated rat lungs (Stanbrook and McMurtry 1983). The Krebs cycle also may be slowed by the inhibition of glycolysis. The glycolysis blockers iodoacetate and 2-deoxyglucose, as well as glucose-free perfusate, augment hypoxic pulmonary vasoconstriction but not vasoconstrictor responses to angiotensin II or KCl in the isolated rat lungs (Stanbrook and McMurtry 1983, Rounds *et al.* 1981). This effect can be prevented by pyruvate, suggesting that the inhibition of glycolysis exerts its influence on the pulmonary vascular tone by decreasing the supply of substrates to the Krebs cycle. It should be noted, however, that Wiener and Sylvester (1991) found hypoxic pulmonary vasoconstriction to be potentiated, even though slowed off, by hyperglycemia.

The diminution of the electron flow through cytochrome aa_3 will have several consequences. Electrons will be taken from the Krebs cycle more slowly; $[NADH]/[NAD^+]$ ratio will rise. Proton gradient across the inner mitochondrial membrane will not be sustained properly. As a result, the phosphorylation of ADP by F_0F_1-ATPase will be limited. It is not clear which of these events might be substantial for pulmonary vasoconstriction elicited by the respiratory chain slowdown.

Administration of 2,4-dinitrophenol, which acts as a proton channel, diminishes the electrochemical gradient of hydrogen ions across the inner mitochondrial membrane. Nonetheless, the flow of electrons through the respiratory chain as well as $[NADH]/[NAD^+]$ ratio may not be influenced. 2,4-dinitrophenol causes pulmonary vasoconstriction, which is very similar to that elicited by the

respiratory chain blockers (Bergofsky *et al.* 1963, Lloyd 1965, Rounds and McMurtry 1981). It suggests that NADH concentration changes are, presumably, not crucial in regulation of pulmonary vascular tone.

An attenuated proton pumping from mitochondria due to the respiratory chain blockade might somehow enhance cytoplasmic pH, at least in the very proximity to the mitochondrial surface. Experimental intracellular alkalosis potentiates hypoxic pulmonary vasoconstriction (Raffestin and McMurtry 1987). As this hyperreactivity is not specific for hypoxic stimuli, the changes in intracellular pH are probably not a substantial part of oxygen sensing mechanism. They may, rather, affect the contractile molecules of vascular smooth muscle.

By reducing the proton gradient across the inner mitochondrial membrane, the slowdown of the respiratory chain impairs the phosphorylation of ADP. If ATP consumption is constant, this decreases intracellular [ATP]/[ADP][P_i] ratio. The attenuation of [ATP]/[ADP][P_i] ratio is a powerful stimulus for cytochrome aa_3 (Erecinska and Wilson 1982). The activation of cytochrome aa_3 by a decrease of [ATP]/[ADP][P_i] ratio augments the flow of electrons through the respiratory chain. By this mechanism, the rate of ATP production can remain unchanged during the action of factors affecting the cytochrome aa_3 activity. The enhanced ATP production tends to improve [ATP]/[ADP][P_i] ratio. The increase of the [ATP]/[ADP][P_i] ratio, however, would abolish the stimulation of cytochrome aa_3 and [ATP]/[ADP][P_i] ratio would drop again. Consequently, [ATP]/[ADP][P_i] ratio will stabilize at a new value, which will be lower than the initial one (Erecinska and Wilson 1982). Therefore, there is no measurable decrease in ATP concentration in hypoxia (Chance *et al.* 1974, Fisher *et al.* 1976, Paul 1989). The cost, however, is a decline of [ATP]/[ADP][P_i] ratio. This ratio was shown to be a function of Po_2 throughout the entire physiological range (Nuutinen *et al.* 1982). It is possible that the [ATP]/[ADP][P_i] ratio may govern some enzyme important for vasoconstriction in a similar manner as it influences the activity of cytochrome aa_3. The evidence for such a link between energy metabolism and pulmonary vasoconstriction is, however, lacking.

Changes in ATP concentration in muscle cells are balanced not only by [ATP]/[ADP][P_i] ratio but also by a creatine/phosphocreatine system. Archer and colleagues (1989d) found that hypoxic pulmonary vasoconstriction was not changed in the rats whose lung creatine content had been lowered by dietary β-guanidino propionic acid. Even though the amount of phosphocreatine in the lungs was not assessed, this study suggests that the creatine/phosphocreatine system is probably not crucial in the mechanism of hypoxic pulmonary vasoconstriction.

The [ATP]/[ADP][P_i] ratio is influenced not only by the rate of ATP formation, but also by the rate of ATP utilization. Therefore, if a cellular energy state is an important link between hypoxia and pulmonary vasoconstriction, the changes in ATP consumption should affect hypoxic pulmonary vasoconstriction. This was the rationale of experiments with ion pumps. The Na^+/K^+-ATPase consumes as much as one third of energy produced in the cell and its inhibition should enhance the intracellular [ATP]/[ADP][P_i] ratio. The Na^+/K^+-ATPase inhibitor, ouabain, blunts vasoconstrictor responses of isolated lungs to hypoxia. By contrast, hypoxic pulmonary vasoconstriction is potentiated by aldosterone, a stimulator of Na^+/K^+-ATPase (Herget and McMurtry 1985). Similarly, hypoxic pulmonary vasoconstriction was reduced by tolbutamide, which inhibits

ATP-sensitive potassium channel. The stimulator of this ATP-consuming pump, diazoxide, mimics hypoxic pulmonary vasoconstriction (Robertson *et al.* 1989).

Glycolytic production of ATP may prevent too deep fall in [ATP]/[ADP][P_i] ratio during the hypoxic suppression of the respiratory chain. Findings of hypoxic pulmonary vasoconstriction potentiated by inhibitors of glycolysis (Stanbrook and McMurtry 1983) and blunted by addition of glucose to hypoglycemic perfusate (Rounds *et al.* 1981) therefore indirectly support the "energy" hypothesis of hypoxic pulmonary vasoconstriction.

The main problem of the "energy" hypothesis of oxygen sensing in lung vessels is that all supporting evidence is indirect. It is based mostly on effects of various inhibitors, the specificity of whose is, generally, low. In addition, there are observations that seems to contradict this hypothesis. Some blockers of cytochrome aa_3 may cause pulmonary vasodilation (Duke and Killick 1952, Sylvester and McGowan 1978).

Conclusion

In conclusion, it should be noted that there are potential interconnections among the cytochrome P-450, oxygen radicals, and "energy" hypotheses (Archer *et al.* 1989a). Activation of cytochrome P-450 results in generation of superoxide anion (White and Coon 1980). The actions of cytochrome P-450 on the vascular tone thus might be related to the redox alteration of Ca^{2+} channels by oxygen radicals. Changes in oxidative phosphorylation alter [NADPH] concentration, which is necessary for regeneration of main cellular antioxidant glutathione. This, as well as oxygen radicals produced in the course of oxidative phosphorylation (by NADH dehydrogenase and at ubiquinone-cytochrome b region) (Turrens *et al.* 1982), offers a potential link between the "energy" hypothesis and a redox state of calcium channels and a subsequent Ca^{2+} influx into the vascular smooth muscle.

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