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Review

Influence of Hyperbaric Oxygen on Blood Vessel Reactivity: Concept of Changes in Conducted Vasomotor Response

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A B S T R A C T

The actions of oxygen in the body are extremely complex, and are also involved in various signalling pathways. Hyperbaric oxygen is known to contribute to the improvement of conditions where tissue circulation is suboptimal, and has considerable usage in different treatment protocols and experimental investigations. However, the precise mechanism by which hyperbaric oxygen changes the functioning of coordinated blood vessel systems and microcirculation is still unknown. Taking into account the known facts, we suggest that hyperbaric oxygen induces changes in conducted vasomotor responses, and in that way influences vascular sensitivity and reactivity to vasodilators and vasoconstrictors. Conducted vasomotor responses are constrictions and dilations that are propagated along the vessel, leading to changes in vessel diameter on a certain distance of the initial site of vasoactive substance activity. Because these vascular responses are of substantial significance in physiological processes, their modification would subsequently cause alterations of blood vessel function and tissue perfusion that could explain observed effects of hyperbaric oxygen. We also discuss potential molecular targets of hyperbaric oxygen, investigation of which could presumably help in the eventual clarification of hyperbaric oxygen action.

***Key words**: hyperbaric oxygen, vascular function, conductive vasoresponse*

Introduction

When oxygen is delivered under high pressure, it increases arterial and tissue pO_2 in the body. This increased pO_2 has been observed to have beneficiary effects on many conditions where reduced tissue oxygenation does not meet the metabolic needs. This therapeutic property is used in *hyperbaric oxygen therapy* to effectively treat various tissue damages. It is becoming clear that the actions of oxygen are very complex – not just explainable as the actions of a nutrient, but as a factor involved in an elaborate system of signalling pathways and capable of changing the expression of proteins, such as growth factors[1]. Hyperbaric oxygen is shown to contribute to the healing of ischemic ulcerations in diabetic patients and to improve outcome of stroke[2], myocardial infarction[3], acute peripheral extremity ischemia in humans[1] and reduced atherosclerotic plaques[4] in animal experimental models[2]. The results of hyperbaric oxygen therapy are clearly documented in experiments and clinical tests, but our understanding and available data of how exactly hyperbaric oxygen exhibits its effects is still very incomplete. We know that the function and structure of resistance vessels is the crucial factor in regulating regional blood flow and oxygen delivery to peripheral tissues. However, studies on the influence of hyperbaric oxygen on vascular function and structure are scarce.

We previously hypothesized that hyperbaric oxygen will modulate mechanisms of vascular responses and vessel-sensitivity to various vasoconstrictor and vasodilator molecules (metabolites of arachidonic acid, nitric oxide), thus improving vascular function and blood flow autoregulation in diabetes mellitus[5]. Recent experimental work showed that microvascular dysfunction in diabetes mellitus is a result of changes in *conducted vasomotor response*[6]. Since hyperbaric oxygen can improve lesions in diabetes (and also other conditions, where vascular damage plays an important role), we propose a concept in which hyperbaric oxygen improves vessel-sensitivity by

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altering conducted vasomotor responses in arterioles. By improving conducted vasodilation and vasoconstriction, it would lead to restoration of vascular function and thereby to better function of the microcirculation. Our theoretical concept might be an important step in the explanation of the biological effects of hyperbaric oxygen on tissue and circulation.

Conducted Vasomotor Response

Localized application of vasoconstrictors and vasodilators on arteriolar surface induces not only a local vasomotor response but also a vasoconstriction or -dilation which is propagated up- and downstream along the vessel. This remote effect of vasoactive substances applied to defined areas of the microcirculation is called *conducted vasomotor response*[7]. It seems that conducted vasomotor response has an important role in physiological processes, e.g. conducted vasodilation of skeletal muscle feed arterioles leads to the development of functional hyperaemia in exercise[8], conducted vasoconstriction is believed to be responsible for upstream contraction of blood vessels in the tubuloglomerular feedback mechanism in the kidney. But it has also been found that conducted vasomotor responses are altered in pathological conditions associated with microvascular dysfunction, including arterial hypertension and sepsis[7].

The elaborate mechanisms underlying this interesting phenomenon have been extensively studied in the last years. Experiments of conducted vasodilation on hamster feed arteries elucidated two distinct and complementary signalling pathways through which the endothelium coordinates relaxation of smooth muscle cells along the wall of resistance microvessels[9]. The first one is electromechanical (electrotonic) conduction of vasodilation and is associated with the spread of hyperpolarization (or depolarization in case of conducted vasoconstriction) along the endothelium and into smooth muscle cells trough *gap junctions*[9,10]. Endothelial cells in blood vessel walls are electrically and chemically coupled and gap junctions (intercellular protein channels connecting the cytoplasm of neighbouring cells) play an important role [7]. The second, much slower pathway, is pharmacomechanical relaxation of smooth muscle cells mediated by Ca^{2+} waves and the release of autacoids (nitric oxide, metabolites of arachidonic acid) along the endothelium. The autors describe these pathways with their 'integrated model of conducted vasodilation'[9]. Activation of membrane receptors (with dilators such as acetylcholine) leads to an increase in intracellular Ca^{2+} concentrations. This increase activates ionic channels producing hyperpolaryzation that travels rapidly along the endothelial cell layer and into smooth muscle cells through myoendothelial gap junctions and causes *electromechanical* conducted vasodilation. The increase in endothelial Ca^{2+} also triggers Ca^{2+} waves, which travel more slowly along the endothelium, trough gap junctions, and activate eNOS (nitric oxide synthase) and cyclooxygenase, producing nitric oxide and prostaglandins. These autacoids diffuse to smooth muscle cells and induce *pharmacomechanical* vasodilation, which sustains the conducted response.

Adenosine triphosphate (ATP), a known vascular regulator of cerebral circulation, is a factor that triggers conducted vasomotor responses. It acts via purinergic receptors on endothelial cells, inducing initial local constriction and subsequent dilation, which is preceeded by membrane hyperpolarization. Nitric oxide and especially cytochrome P450 metabolites of arachidonic acid (epoxyeicosatrienoic acids) have been found to mediate dilations caused by ATP[11].

The Concept of Hyperbaric Oxygen Influence on Vessel Reactivity

The exact molecular and cellular mechanisms by which hyperbaric oxygenation evokes its experimentally observed effects have not been identified yet. In the light of the known facts and information about conducted vasomotor responses and the visible benefits of hyperbaric oxygen, we propose that hyperbaric oxygen evokes its actions by inducing *changes in conducted vasomotor responses*. With improved conducted vasomotor responses, arterioles that control local blood flow, resistance, microcirculation and thereby transport of nutrients to cells, will be able to react more appropriately to tissue demands and regulate perfusion, i.e. will have restored function. Since conducted responses have been shown to be an important factor in physiological regulation of circulation (as mentioned earlier), effective conducted responses of vessels to vasodilators or vasoconstrictors (that are signaling tissue needs) can have a crucial impact on conditions (including pathological) where tissue function, endurability and survival are challenged. In this regard, an improvement of conducted responses in conditions where these vascular responses are damaged, would lead to an enhancement of the status (and such enhancements have been observed with hyperbaric oxygen therapy in conditions with such damages – see later). There are some experimental indications of effects of hyperbaric oxygen on vascular motorics and reactivity[12,13], and we suggest that the way it exerts these effects is by influence on conducted responses.

Taking into account the molecular interactions involved in conducted vasomotor responses, there are several hypothetical mechanisms by which hyperbaric oxygenation may cause changes. We are presenting these proposed mechanisms in Table 1 and hold that one or more of them can play a crucial role in the influence of hyperbaric oxygenation.

Because gap junctions have a central function in the regulation of conducted vasomotor responses, changes in expressions of their components, changes in the conformation of their proteins or indirectly transmitted regulative signals like phosphorylation would cause substantial changes in vascular reactivity. High concentrations of oxygen produce free radicals and other reactive molecules called reactive oxygen species which can act as signaling

TABLE 1
HYPOTHETICAL MECHANISMS AND POSSIBLE TARGET CANDIDATES' OF HYPERBARIC OXYGENATION INFLUENCE ON CONDUCTED VASOMOTOR RESPONSES

Modification	Description	Impact
Gap junction upregulation	Increased expression of connexins (gap junction proteins) leads to increased number of gap junctions	Better spread of hyper-/depolarization and Ca^{2+} waves
Certain DNA segments	Influence of po2 on expression regulation via transcription factors (e.g.*hypoxia-inducible factors*)	Change of expression of various proteins
Protein conformation	Changes of protein conformations of various proteins, either directly or indirectly (e.g.via ros)	Functional changes that influence possibly receptors, ionic channels, gap junctions
Membrane changes	Changes of memrane potential, channels, receptors, phospholipids, directly or indirectly	Influence on hyper-/depolarization and signal transduction
Oxygen sensors (CYP450-4A)	Formation of metabolites of CYP450	Metabolites of CYP450 have an important role in conducted vasomotor responses
Formation of ROS	Increased oxygenation leads to formation of highly reactive free radicals (O_2^-, OH^-) which interact with multiple macromolecules	Changes of proteins, phospholipids, nucleic acids and influence on antioxidant systems
ATP	ATP synthesis depends directly on the amount of oxygen	ATP has a significant role in conducted vasomotor responses
NO	hyperbaric oxygen causes changes in NO formation (influence on eNOS)	NO is an important factor in mechanisms of conducted vasomotor responses

ROS – Reactive oxygen species, NO – Nitric oxide, ATP – Adenosine triphosphate, eNOS – Endothelial nitric oxide synthase

molecules in vascular physiology and pathophysiology[14], serving as second messengers to activate multiple intracellular proteins and enzymes. Hyperbaric oxygen can lead to changes in protein expression. It has been found that it can influence transcription factors such as *hypoxia-inducible factor 1*[15,16] and expression of proteins like growth factors[1]. There is evidence of oxygen sensor existence – a cytochrome P450 enzyme of the 4A family of omega-hydroxylases can, by generating metabolites of arachidonic acid (20-HETE), adjust arteriolar diameter depending on pO_2[17]. Since cytochrome P450 and metabolites of arachidonic acid are also very important in the mechanism of *conducted* vasomotor responses, these facts strongly go in favour of our concept of hyperbaric oxygen impact on conducted responses. Synthesis of ATP directly depends on oxygen, and nitric oxide production has been linked to hyperbaric oxygen[18,19,20]. Both of these factors are known to modify conducted vasomotor responses and are therefore plausible connections between hyperbaric oxygen and conducted responses. It is likewise interesting that nitric oxide can regulate gap-junctional coupling of some types of cells (experiments with HeLa cells and neural connexins) through indirect triggering of connexin phosphorylation[22]. These known facts, therefore, support several of our proposed mechanisms and we think that hyperbaric oxygen may exert its effect through a complex system of signaling and regulatory processes which act simultaneously and are interconnected. In this context, oxygen would activate more then one mechanism and these mechanisms would ultimately lead to the same end result – changes in conducted vasomotor responses of blood vessels, enhanced blood vessel reactivity and thereby better and more accurate vasodilation or vasoconstriction as an answer to tissue needs.

Evaluation of the Concept

There is a number of experimental studies with results and conclusions that would fit into this presented concept, many of them already mentioned while elaborating it. Benefits of hyperbaric oxygen therapy in pathologic conditions that have disruptions of conducted vasomotor responses as the basis of vascular dysfunction, represent strong support of the possibility of it affecting vascular sensitivity and reactivity (and thereby function) through changes in conducted vasodilatory or vasoconstrictive responses to tissue regulating factors. Research done on mice with streptozotocin – induced diabetes showed decreased conducted vasoconstrictor response in cremaster arterioles. Microvascular disease is a common and severe complication in diabetes, and these findings suggest a role of conducted responses in the pathophysiology of microvascular dysfunction[6]. The fact that hyperbaric oxygenation showed success in treatment of diabetic complications and chronic wounds,[22–24] in concordance with the concept. It is intriguing that chronic intermittent hypoxia impairs vascular relaxation responses similar to diabetes mellitus[25], what raises the

question of oxygen potentially having the opposite effect on vessels. Microvascular dysfunction in sepsis as well has been linked to altered conducted vasomotor responses[7] and hyperbaric oxygen was found to have therapeutic effects in sepsis[26,27]. Hyperbaric oxygen can improve outcome of stroke[2], but it has also been demonstrated that it can change cerebral blood flow after global cerebral ischemia[28]. Its potential to change blood flow is another support of this concept because conducted vasomotor responses contribute to the control of major feed arteries, and by that means to the blood flow of a given tissue.

Experiments on anaesthetized artificially ventilated dogs indicated that hyperbaric oxygen can have an effect on vasomotor tone in acute intracranial hypertension and induce changes of cerebral vessel reactivity to CO_2[12]. In our opinion, it is possible that the way hyperbaric oxygen causes this is by altering conducted vasomotor responses. Furthermore, hyperbaric oxygen has positive influences on vascular reactivity and mean arterial pressure[13] in rats treated with zymosane (which normally causes vasoplegic response and vascular derangement and is used to create an experimental model of multiple-organ-failure syndrome). In a rat transient focal cerebral ischemia model, alteration of cyclooxygenase-2 expression was involved in hyperbaric oxygen treatment[2]. The results showed that hyperbaric oxygen applied at six hours after reperfusion significantly reduces infarct area as compared with no-treatment group. Interestingly, cyclooxygenases, and their metabolites – prostaglandins, have a prominent role in the integrated model of conducted vasodilation of Domeier and Segal.

For better understanding of the mechanisms by which hyperbaric oxygen acts in the microenvironment and how it leads to functional and signaling alterations that could be held accountable for its visible effects, further research is necessary. We think that investigations should pursue a possible connection between pO_2 and conducted vasodilation/constriction and seek more evidence for potential molecular interactions that are proposed in this concept. Experiments on rat or mouse cremasters, for instance, would be interesting because of the possibility to directly visualize and measure functioning blood vessels *in vivo*. Groups of animals treated with hyperbaric oxygen could be compared with controls. Cremaster arterioles in these groups can be locally stimulated with vasoactive substances and the resulting local and conducted vasodilation or vasoconstriction can be measured. Also, animal models of various conditions where conducted vasomotor responses have been found to be disrupted (such as diabetes or sepsis) could be used to study the effect of hyperbaric oxygen on conducted vasomotor responses. Differences between such models and healthy animals would be particularly interesting for the confirmation of this hypothetical concept, and would have important implications for the future, including for the use in therapy. Similar experiments could be done *in vitro*, with isolated blood vessels in perfusion chambers and measurement of conducted response after application of vasoactive substances.

Consequences and Concluding Remarks

If confirmed, these mechanisms would represent a significant advancement in the interpretation of biological changes at molecular, cellular and functional levels after hyperbaric oxygenation. Better understanding of events at the site of action would enhance the use of hyperbaric oxygen therapy, more clearly define and expand the indications and precisely establish limits and contraindications. But except to improvements in therapy, for what more knowledge is essential, it will also lead to a wider view on oxygen as a signaling agent and it will have implications for further theoretical constructs regarding the general functions and roles of oxygen in biological systems. From today's viewpoint, it is clear that the visible effects of hyperbaric oxygen can not be explained merely as a consequence of improved transport of oxygen as a nutrient to tissues, and its utilization in metabolic pathways. There is evidence that oxygen has a much broader and more significant function, and that it is an important signaling factor involved in signaling pathways[1]. The effect on conducted vasodilation and vasoconstriction as the mechanism of vessel reactivity-changes is probably not the only way hyperbaric oxygen acts on vasculature. Its interactions with the circulation are very complex, hardly explainable with one mechanism, but an intertwined system of incisively regulated processes.

On the one hand, a significant part of this system may be the impact of oxygen as a signaling factor, influencing vascular reactivity through a complicated set of interacting mechanisms which we focused on in this proposed concept. On the other hand, there are the actions of oxygen as a nutrient, as a modulating agent in metabolic processes, as a factor leading to changes of other regulating systems (which again interact with vasculature and circulation), as a toxic agent (leading to oxidative stress) and as a cause of other physical changes in the living organism, which are certainly not negligible. All of this is making the role of oxygen very interesting, but also very complicated, and the complete understanding of it very challenging. However, stepwise investigation might result in the elucidation of these interactions, and this concept can contribute as one of the pointers in this undertaking.

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UTJECAJ HIPERBARIČNOG KISIKA NA REAKTIVNOST KRVNIH ŽILA: KONCEPT PROMJENA U PROVODNIM VAZOMOTORNIM ODGOVORIMA

SAŽETAK

Djelovanja kisika u tijelu su iznimno kompleksna i uključena su u razne signalne putove. Poznato je da hiperbarični kisik pridonosi poboljšanju stanja u kojima tkivna cirkulacija nije optimalna te se koristi u različitim protokolima liječenja i u eksperimentalnim istraživanjima. Međutim, točni mehanizmi s kojima hiperbarični kisik mijenja koordinirani sustav krvnih žila i mikrocirkulaciju još nisu poznati. Uzimajući u obzir poznate podatke, mi predlažemo koncept prema kojemu hiperbarični kisik uzrokuje promjene u provodnim vazomotornim odgovorima i na taj način utječe na vaskularnu senzitivnost i reaktivnost na vazodilatatore i vazokonstriktore. Provodni vazomotorni odgovori su konstrikcije i dilatacije koje se prenose uzduž žile, dovodeći do promjena u žilnom promjeru na određenoj udaljenosti od početne lokacije vazoaktivnog podražaja. S obzirom da su ti vaskularni odgovori od velikog značaja u fiziološkim procesima, njihova modifikacija bi napsoljetku uzrokovala promjene funkcije krvnih žila i tkivne perfuzije koje bi mogle objasniti primijećene efekte hiperbaričnog kisika. Također ćemo raspraviti potencijalne molekularne mete hiperbaričnog kisika, čije bi daljnje istraživanje moglo pomoći u konačnom objašnjenju djelovanja hiperbaričnog kisika.