Metabolic Control of Microvascular Networks: Oxygen Sensing and Beyond

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Key Words

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Abstract

The metabolic regulation of blood flow is central to guaranteeing an adequate supply of blood to the tissues and microvascular network stability. It is assumed that vascular reactions to local oxygenation match blood supply to tissue demand via negative-feedback regulation. Low oxygen (O_2) levels evoke vasodilatation, and thus an increase of blood flow and oxygen supply, by increasing (decreasing) the release of vasodilatory (vasoconstricting) metabolic signal substances with decreasing partial pressure of O_2 . This review analyses the principles of metabolic vascular control with a focus on the prevailing feedback regulations. We propose the following hypotheses with respect to vessel diameter adaptation. (1) In addition to O_2 -dependent signaling, metabolic vascular regulation can be effected by signal substances produced independently of local oxygenation (reflecting the presence of cells) due to the dilution effect. (2) Control of resting vessel tone, and thus perfusion reserve, could be explained by a vascular activity/hypoxia memory. (3) Vasodilator but not vasoconstrictor signaling can prevent shunt perfusion via signal conduction upstream to feeding arterioles. (4) For low perfusion heterogeneity in the steady state, metabolic signaling from the vessel wall or a perivascular tissue sleeve is optimal. (5) For amplification of perfu-

sion during transient increases of tissue demand, red blood cell-derived vasodilators or vasoconstrictors diluted in flowing blood may be relevant.

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Introduction

More than 200 years ago, John Hunter found it 'a common principle in the animal machine, that every part increases in some degree according to the action required. Thus we find...vessels become larger in proportion to the necessity of supply ...; the external carotids in the stag, also, when his horns are growing, are much larger than at any other time' [1]. It is now accepted that vessels are dynamic structures, which are controlled in structure and function by hemodynamic and biological signals related to cell metabolism, a process which may be called 'angioadaptation' including angiogenesis, pruning, remodeling and changes in vascular tone [2, 3]. This vascular plasticity stimulates the question as to the mechanisms providing vascular reactions to match substrate supply by the blood to tissue metabolic demand in terminal vascular beds and their functional implications.

In metabolic control, oxygen (O_2) is usually assumed to play a central role. A mismatch of O_2 supply relative to tissue demand can result from a multitude of conditions, including decreased oxygenation of the blood, reduced or heterogeneous perfusion, or an increase of

metabolic tissue needs. For each of these conditions, there is ample experimental evidence of metabolic signaling and involved mechanisms. Most of the respective studies address the acute modulation of vascular tone, while much less is known about the long-term adaptation of vascular structure due to experimental limitations. The current knowledge about metabolic control under conditions of tissue hypoxia and/or early and sustained phases of exercise is summarized in many excellent reviews [4–13]. So this article does not aim at providing a comprehensive literature review, but rather attempts to integrate these experimental and theoretical findings in order to derive more general principles of the local metabolic control of terminal vascular beds, with a focus on the control of vessel diameter and the interrelations between vascular reactions on the different time scales. We will also comment on changes in vessel numbers by angiogenesis/pruning.

Obviously, individual mechanisms of metabolic control are of varying significance in different organs as they are modified and complemented by tissue-specific effects reflecting local needs and conditions. Under conditions of increased tissue metabolic demand, feed-forward regulations could be established by metabolic signaling related to O_2 turnover. Local metabolic regulation also acts in concert with neural, humoral and hemodynamic influences to evoke vascular reactions under in vivo conditions, which change under non-steady-state conditions (e.g. during physical exercise) in a time-dependent manner. Moreover, the relative impact of local metabolic signals on vascular adaptation will change along with changes in the global state of the organism. These aspects as well as vascular adaptation under the pathophysiological conditions associated with general or local hypoxia are beyond the scope of this review.

We will discuss general concepts of metabolic vascular control derived for a systemic (i.e. not lung-like) tissue with ‘average’ properties under physiological conditions. Additions may be needed in certain tissues according to the specific condition. We specifically (1) call attention to a mechanism allowing metabolic signaling independent of O_2 and other indicators of the supply-to-demand ratio, (2) propose a concept for the adjustment of the balance between vascular resting tone and structural vessel diameter determining perfusion reserve in a vessel, (3) compare the suitability of vasodilatory and vasoconstricting metabolic signal substances for guiding vessel diameter adaptation and (4) propose different roles for O_2 sensors located in vessel walls, red blood cells (RBCs) and tissue cells.

Current Concepts of Metabolic Vascular Regulation

Feedback Loops via Local Signals and Information Transfer

Microvessels continually adapt their diameters to the surrounding conditions (fig. 1). Theoretical considerations have shown that vessel diameter adaptation to hemodynamic signals alone evokes a maldistribution of blood flow and microvascular network instability via positive-feedback regulation [14, 15]. According to biological experiments, an increase in shear stress stimulates vessel enlargement [16]. If two vessels are connected in parallel (thus experiencing the same pressure drop), the vessel with higher flow also exhibits higher wall shear stress which will evoke an increase in diameter, further increasing blood flow in this vessel. In the parallel branch, the opposite reaction is evoked, reducing complex vascular networks to single arterio-venous shunts.

Adaptive signals reflecting local metabolic conditions can counteract these destabilizing effects by providing negative-feedback regulation and are thus essential to maintain parallel flow pathways [17]. Such negative-feedback regulation via metabolic signaling was proposed by Berne [18] and Gerlach et al. [19] in 1963 for short-term metabolic diameter regulation in the coronary circulation. Reduction of the local partial pressure of oxygen (pO_2) by hypoxemia, decreased blood flow or increased O_2 demand leads to the release of metabolic signal substances (e.g. adenosine, but probably others as well, as described below) from parenchymal cells which diffuse to the vessel wall and evoke vasodilatation. The resultant flow increase elevates O_2 pressure, in turn reducing the production rate of the metabolic stimuli.

To prevent functional shunting of perfusion, local metabolic information also has to be transferred downstream via the convection of metabolic signal substances and upstream via conduction along the vessel wall [20–24]. Conduction and shear stress-mediated dilatation based on hemodynamic coupling in microvascular networks [25] are also critical for the transfer of information from the postcapillary and venous vessels, that receive most of the metabolic signals, to the arterioles which control flow resistance and perfusion [26, 27]. These processes may be supported by veno-arteriolar diffusion of metabolic signal substances [28, 29]. Hemodynamic and metabolic signals evoke adaptive stimuli which are constantly and simultaneously acting on any given vessel (see fig. 1a), albeit with different combinations for different vessels and with net changes in response to changes in tissue conditions [17].

O₂ Sensitivity and Perfusion Control at Rest and with Increased Metabolic Demand

Several ideas about the way low O₂ levels induce vasodilatation by negative-feedback regulation have been proposed for resting conditions including: (1) the increasing release (or decreasing destruction/inhibition) of metabolic signal substances with decreasing pO₂ causing vasodilatation, (2) the diminishing release (or increasing destruction/inhibition) of vasoconstricting signal substances with decreasing pO₂ and (3) the direct effects of O₂ on smooth-muscle cells (and/or endothelial cells), with decreasing pO₂ causing smooth-muscle relaxation [30–36].

These mechanisms may be supplemented by further effects under conditions of enhanced metabolic activity, e.g. during physical exercise. Vasodilatory metabolites secreted from tissue cells at a rate proportional to oxidative metabolism (e.g. carbon dioxide and reactive oxygen species) may contribute to the metabolic control of vessel diameters via feed-forward control [37, 38]. Additional O₂-independent processes may also be involved in evoking the vast blood flow increase that can be observed. These processes may include mechanical interactions between the contracting skeletal muscle and the vasculature [39], neural effects including sympathetic withdrawal and active vasodilatation (partially in a feed-forward manner) via sympathetic β_2 vasodilator fibers (e.g. in the heart) [40, 41] and possibly also vasodilatation elicited by acetylcholine spillover from active neuromuscular junctions in some species, especially for the rapid onset of vasodilatation [42, 43]. As a result of local metabolic, neural or humoral feed-forward mechanisms, the supply-to-demand relation in light exercise may even be improved.

Metabolic Signal Substances

Based on experimental studies of acute vessel diameter adaptation, numerous metabolic signal substances released from various sources (fig. 1b), i.e. the parenchymal tissue, the vessel wall and RBCs, have been implicated in coupling blood flow to local oxygenation and contractile activity.

Vasodilators

- Parenchymal tissue cells, representing the ‘end of the oxygen supply chain’, are usually considered to play a leading role in metabolic regulation. Several metabolic signal substances increasingly produced/released with decreasing pO₂ have been suggested. (1) Under resting conditions and hypoxemia (systemic hypoxia) a number of studies have shown the involvement of adenosine [12]. Others have reported

Fig. 1. Adaptation stimuli and origin of metabolic signal substances. **a** Schematic drawing of local adaptation stimuli and intervascular information transfer. Any vessel is exposed to metabolic stimuli (green arrows) and hemodynamic stimuli (blue arrows) evoking vessel diameter adaptation (simultaneous action of humoral, neural and interstitial mechanical stimuli not shown). Metabolic stimuli acting locally are, for example, derived from the local pO₂. Information on local metabolic conditions is transferred upstream by conduction via gap junctions in the vessel wall, downstream by convection of metabolic signal substances in the flowing blood and possibly from veins to arteries by diffusion, thus contributing to diameter adaptation in remote vessels. In addition, hemodynamic signals (e.g. transmural pressure and shear stress) evoke diameter adaptation locally, and vessels are hemodynamically linked within the network via hemodynamic coupling. **b** Schematic drawing of possible origins of metabolic signal substances. Local O₂ levels (red arrows show O₂ transport) evoke the release of metabolic signal substances (green arrows) from the vessel wall, RBCs and tissue cells.

that adenosine appears to contribute only during hypoperfusion not hypoxia [44].

(2) During enhanced metabolic demand, and depending on the actual conditions, tissue cells release several substances that apparently act as vasodilatory mediators, such as the classical ‘muscle activity signals’ [4] including increased pCO₂, lactate, K⁺ and adenosine, but also cytokines (e.g. IL-6) possibly acting via neuronal nitric oxide synthase (NOS)-derived nitric oxide (NO), prostaglandins (PGI₂ and PGE₂), H⁺, inorganic phosphate and reactive oxygen species; osmolality itself may also evoke vasodilation [18, 45–49]. Only recently, adenosine triphosphate (ATP), at

the interstitial concentrations reached in physical exercise, was suggested to play a role [50].

Alternatively, vasodilatory substances released at a constant rate at a low pO_2 (including NO) may be suppressed, degraded or neutralized at a high pO_2 [51, 52].

- An O_2 sensor located in the vessel wall or close to it would be well-suited to guide vessel diameter adaptation because both hypoxemia and an increase in the tissue metabolic rate of O_2 result in a decreased pO_2 in the vessel wall [53–56]. Experimental studies also show that aparenchymal arteriolar segments in vivo retain their functional integrity including O_2 sensitivity [20, 57]. Various potent vasodilators are produced by endothelial cells in direct response to a low pO_2 , including NO produced by endothelial NOS or released from smooth-muscle cell myoglobin, prostaglandins, endothelium-derived hyperpolarizing factor and adenosine [47, 58–66]. The role of NO and prostacyclin in O_2 -sensing by the vessel wall, however, is difficult to assess because the release of these substances is also affected by other vasoactive substances (e.g. adenosine, ATP, acetylcholine and bradykinin) and by hemodynamic signals (e.g. shear stress) [5].
- In the last decades, RBCs were suggested to act as mobile O_2 sensors. Three regulatory mechanisms based on metabolic signaling in response to the O_2 saturation (sO_2) of hemoglobin (Hb- sO_2), and thus also in response to local pO_2 due to the monotonic, non-linear coupling of both parameters by the O_2 -binding function, have been put forward: (1) the release of ATP in response to low Hb- sO_2 , which, together with its breakdown products adenosine diphosphate and adenosine monophosphate, induces vascular smooth-muscle relaxation [67–71], (2) the release of vasodilatory NO stored in RBCs as S-nitrosohemoglobin [72, 73] and (3) the release of NO via reduction of nitrite [74–79]. The possible impact of NO released from RBCs (and cell-free hemoglobin) in causing hypoxic vasodilatation in vivo was analyzed using a multi-cellular computer model [80, 81].

Vasoconstrictors

Recently, an alternative concept for metabolic signaling has been suggested based on experimental data entailing a maximal production of vasoconstricting substances at a high pO_2 which is increasingly suppressed at declining O_2 levels [82–90]. The mechanisms of vasoconstrictor effects exhibit significant regional heterogeneity [82, 88]. Lipoxygenase products such as leukotrienes [83, 85, 88] and 20-hydroxy-eicosatetraenoic acid (20-HETE) [82,

84, 86, 87, 90], an ω -hydroxylation product of arachidonic acid produced by the CYP450-4A enzyme system in the presence of molecular O_2 , were observed to evoke vasoconstriction in an O_2 -dependent manner. 20-HETE is produced in both the arteriolar wall and parenchymal cells [86].

To date, however, the hypothesized impact of individual mechanisms in diameter regulation in vivo remains controversial, and there are regional differences in mechanisms in different tissues. Also, the phenomenon of redundancy of the action of different metabolic signal substances makes it difficult to determine their relative contributions in vivo [11, 12, 91]. For exercise hyperemia, it has been stated that out of a ‘laundry list’ of potential signal substances [11] released under these conditions, ‘there is clearly no single signaling molecule that drives the response’ [4].

Principles of Metabolic Vascular Regulation

Functional Scheme of Metabolic Regulation

In figure 2, we have tried to integrate current knowledge on metabolic signaling into a functional scheme of metabolic regulation. As described before, the current concepts of metabolic regulation of vascular adaptation are based on the assumption that metabolic signal substances are released from parenchymal tissue cells, the vessel wall and/or the RBCs [56]. For a principal analysis of metabolic regulation of angioadaptation, figure 2 considers biological ‘tasks’, ‘solutions’, ‘implementations’ and ‘time scales’, analyzed in detail in the following sections.

The main physiological goal for microvascular networks is to provide blood supply to all tissue cells in all functional states. This implies different tasks: sufficiently low diffusion distances for substrates from the vascular lumen to the parenchymal cells, sufficiently homogeneous perfusion under steady-state conditions and the ability to adequately increase perfusion under conditions of transient increases in tissue demand. The biological solutions to meet these tasks include angiogenesis (by sprouting or intussusception) as well as vessel diameter adaptation, both by means of structural, long-term changes (remodeling) and short-term changes in smooth-muscle tone. The physiological tasks are met chiefly via negative-feedback regulation where metabolic signal substances mediate between the metabolic state of the tissue (e.g. hypoxia) and the vascular reaction evoked. In the following paragraphs, we will describe the biological pro-

Fig. 2. Concepts for metabolic vascular feedback control. Hypothesized scheme of metabolic feedback regulations of vascular adaptation: stimulating effects (arrows), inhibitory effects (blunted lines). For better readability, only the vasodilatory (not vasoconstricting) effect of the metabolic signal substance is shown in diameter adaptation (middle and bottom rows). Top: a central task of vascular design is the maintenance of adequately low diffusion distances. This requires angiogenesis elicited by signals generated in response to low O_2 availability in the tissue. Middle: in a vascular network with given number and arrangement of vessels, adequate distribution of perfusion at rest is maintained by adjustment of inner vessel diameters, depending on maximally dilated, structural diameter and superimposed resting tone. Long-term decreased O_2 levels evoke release of metabolic signal substances which lead to structural vessel diameter adaptation. We propose the additional contribution of an O_2 -independent mechanism to metabolic control of vessel diameters, the dilution effect (orange line and font, see also fig. 3): for any signal substance released into the blood at a given rate, the concentration increases with decreasing perfusion.

Vessels with the lowest flow thus exhibit the strongest signal, and a negative-feedback regulation of perfusion is established for signal substances evoking vasodilation. Bottom: transient reductions in tone leading to an increase in diameter are triggered by transient hypoxia, establishing a negative-feedback loop. In addition, a long-term regulation of resting vascular tone is needed to maintain a specific level of flow reserve available for an increase in perfusion as demand increases. For this regulation of the resting tone level, a vascular activity/hypoxia memory is proposed (blue lines and font, see also fig. 4): repetitive episodes of transient tissue activity increase and/or hypoxia trigger a sustained increase in resting tone and flow reserve. In spite of increased resting tone, an adequate resting vessel lumen is maintained by a parallel adaptive increase of structural vessel diameter. Alternatively, the vascular activity/hypoxia memory could evoke an increase in structural diameter, which then would lead to a compensatory increase in resting tone (not shown on graph). Conc MetS = Concentration of metabolic signal substance in the blood; MetS = Metabolic signal substance.

cesses involved with a focus on their relation to metabolic feedback regulation.

The metabolic feedback regulation of angiogenesis is addressed in figure 2 (upper row). The large number of parallel flow pathways observed in vivo is dictated by the

necessity to ensure that the maximal distance between capillaries and the mitochondria of parenchymal cells, the end of the delivery chain, is lower than the effective O_2 diffusion distance. If this condition is not met, the oxygen partial pressure in parenchymal cells is low. Hypox-

ia then elicits the production of diffusible signal substances that have very high effective diffusion distances because (in contrast to O_2) they are not consumed by the tissue. These signals evoke an increase in the number of vessels by angiogenesis, thus reducing the diffusion distance (for review, see [92]). For tissues with a higher specific O_2 demand, the effective O_2 diffusion distance is lower and a higher vessel density results, in accordance with experimental observations. The metabolic sensor for angiogenesis signaling must be in the tissue and not in the vessel wall or in the RBCs because in the case of insufficient vessel density, vascular pO_2 may be high despite tissue hypoxia [56]. To generate functionally adequate network structures, angiogenesis has to work in concert with the refining processes of vascular diameter remodeling and the pruning of abundant vessels [3].

The processes underlying vessel diameter control are described in figure 2 (middle and lower rows; for simplicity, only metabolic signaling by vasodilators is shown). Resting flow, flow distribution and the increase of perfusion during increased demand are controlled by changes in the inner (luminal) diameter of existing vessels in response to metabolic signals on different time scales. The inner vessel diameter is determined by the structural (i.e. fully dilated, passive) vessel diameter and superimposed smooth-muscle tone (i.e. vasoconstriction exerted by smooth muscle cells, most notably in the arteriolar vessel wall). Under long-term, stable resting conditions, diameter adaptation serves to guarantee an adequate bulk flow rate and to overcome the perfusion heterogeneity arising from the inevitable structural heterogeneity of terminal vascular beds [93]. In this regulation, metabolic signals derived from local oxygenation evoke the adjustment of structural vessel diameters to provide, in combination with resting tone, appropriate inner vessel diameters. In case of transient increases of metabolic activity and/or tissue hypoxia, vascular smooth muscle tone is decreased to transiently increase perfusion. The maximal extent of this perfusion increase, i.e. the perfusion reserve, is determined by resting tone level.

We propose that there are two additional mechanisms involved in the metabolic control of vessel diameters.

Dilution Effect

The O_2 -dependent release of metabolic signal substances provides a strong negative feedback that elicits a diameter increase in vessels with a low pO_2 [18, 19], as described before. Here we propose that, in addition to this well-accepted concept, negative-feedback regulation by vasodilators may be elicited even without depen-

dence of signal production on the actual metabolic state due to a 'dilution effect' [fig. 2 (middle row); fig. 3]. For any substance (produced by the tissue, vessel wall or RBCs) that diffuses into the blood stream, the concentration in the blood increases inversely with decreasing blood flow, i.e. the lower the blood flow, the lower the dilution volume and the higher the concentration. If the substance is vasoactive, the stimulus for vessel diameter change is thus selectively higher in vessels with a low flow. For vasodilatory metabolic signal substances, this leads to a functionally required diameter increase in underperfused vessels (negative feedback) even if the substance is released at a constant rate (e.g. in proportion to the presence of cells). For vasoconstricting metabolic signal substances, however, the increasing stimulus in underperfused vessels that results from the dilution effect would promote a further decrease in vessel diameter and perfusion, thus establishing an unwanted positive feedback.

O_2 -independent metabolic signals would allow vessels to effectively 'measure' local perfusion via the dilution effect, analogously to the well-known 'indicator dilution method'. The dilution effect may thus contribute to the metabolic control of supply by regulating the blood flow and the supply of O_2 . As shown in figure 3, the dilution effect seems to be very powerful in this context; even a complete abolishment of O_2 sensitivity in metabolic vasodilatory signaling leads (in the computer simulation considered) to only small, local changes in the distribution of flow and O_2 under resting conditions.

As a consequence, vasodilatory substances which are produced without or with only a limited relation to O_2 levels may be candidate metabolic signal substances for the regulation of vascular structural diameter occurring in any vessel, and such substances may have been overlooked in experimental studies of metabolic vascular regulation. Potential metabolic signal substances would have to be constantly released along the whole network into the blood, and they would have to be able to pass the barrier to diffusion from the lumen to the smooth muscle likely established by the endothelial cell membrane (which is more difficult for water-soluble than for lipid-soluble compounds) [94, 95], and should be able to evoke an effect throughout the network (e.g. via constantly available and homogeneously distributed 'receptors'). While access of the signal substance to receptors in the vessel wall is crucial, the second condition (homogeneous vascular reaction to the signal substance) is rendered less relevant by the presence of signal conduction via connexins (see fig. 1).

Fig. 3. Dilution effect. Both O₂-dependent (top left) and O₂-independent (top right) production of vasodilatory metabolic signal substances (Prod MetSubst) can provide signals for diameter increase of underperfused microvessels: If a signal substance is released into a vessel at a given rate, there will be an inverse relation between intravascular concentration (Conc) of the signal substance in the blood (Conc MetSubst) and blood flow (dilution ef-

fect, middle). The properties of microvascular networks with geometry observed in vivo (lower panels: mesenteric microvascular network, 576 vessel segments) assessed by simulated adaptation [26, 56] with (left) or without (right) O₂ dependence of metabolic signaling are very similar, with the exception of lower O₂ levels (but not hypoxia) observed in some smaller vessels.

It should be noted that a possible independence of metabolic control from the O_2 levels only pertains to the structural adaptation of steady-state inner vessel diameter whereas both the control of angiogenesis and of vascular tone during the transient increase in tissue activity require that metabolic signaling is dependent on local pO_2/O_2 availability in relation to tissue O_2 demand (fig. 2).

Vascular Activity/Hypoxia Memory

Acute changes in local metabolic conditions related to O_2 availability are well known to evoke short-term vessel diameter changes via a change of smooth-muscle tone [55, 96], as described before. However, much less is known about the mechanisms that control the relation between structural (maximally dilated) vessel diameter and superimposed smooth-muscle tone in establishing a given inner vessel diameter. This relation is of importance, since the level of smooth-muscle tone determines the arteriolar dilation reserve and, in consequence, also the arteriolar perfusion reserve. Thus, the level of resting tone [97] has to be adjusted to match the perfusion reserve to the extent of transient increases in demand in a given tissue (fig. 2, lower panel).

Exposure to stimuli resulting from elevated levels of flow or shear stress and pressure, conditions typically occurring in physical exercise, has been shown to evoke an increase of vascular responsiveness to adrenergic, metabolic or hemodynamic signals [98–100]. This so-called ‘vascular conditioning’ [101] would not, however, change the tone level itself but rather increase the extent of tone reduction for a given tone level under conditions of increased demand.

Experimental data on arterioles showed that maintained levels of high tone are transformed into corresponding decreases of structural vessel diameter (inward remodeling) and vice versa [102–104]. This transformation of changes in tone into a structural vessel adaptation would control wall tension and could regulate the level of smooth-muscle tone [105, 106]. Under conditions of persistent change, this transformation will reduce the energy expenditure and is thus an attractive physiological concept. However, over time, vessel tone would always be reduced to a minimal level. Such a general decline in tone is in contrast to the specific needs for perfusion reserve in individual tissues. The more heavily and frequently perfusion demand is increased (e.g. in muscle activity), the more perfusion reserve, and thus a high vascular tone during rest, is required while maintaining an adequate resting perfusion. To generate different levels of resting

tone and dilation reserve in different tissues, additional mechanisms and signals are needed.

Figure 4 specifies the hypothesis on a ‘vascular activity/hypoxia memory’ allowing adjustment of the level of resting tone in a given tissue in response to the extent of transient increases in demand in a given tissue. We hypothesize that the acute vascular reaction to increased activity not only triggers a transient decrease in smooth-muscle tone leading to an acute increase in perfusion, but also determines resting levels of tone via a vascular activity/hypoxia memory. The envisaged mechanism is an increase in resting tone elicited by repetitive episodes of transient activity increase, e.g. during skeletal-muscle work bouts (see fig. 4). The long-term increase in vascular tone elicited by the activity periods and transient hypoxia might be established by long-term changes in gene expression.

At first glance, it may seem paradoxical that in the context of vascular activity/hypoxia memory, repetitive episodes of hypoxia should elicit a sustained increase in resting vascular tone, i.e. in contrast to the well-known direct dilating effect of hypoxia. However, the increased resting vascular tone would be compensated by a parallel increase in structural vessel diameter, maintaining an adequate inner vessel diameter and thus perfusion (fig. 4). The higher resting tone would allow a greater increase in perfusion during subsequent transient phases of increased demand. Alternatively, an opposite direction of the causal chain may be envisaged, with repetitive phases of increased activity/hypoxia leading to a primary increase in structural vessel diameter (i.e. by translating sustained decreases of tone to structural diameter increase [102–104]) which would then trigger a compensatory increase in resting tone, again maintaining the inner vessel diameter. In this way, the vascular memory would be manifested in the structural vessel diameter. In both cases, the response to repetitive transient hypoxia during phases of increased demand would be characterized by an increase in resting smooth-muscle tone and a commensurate increase in maximally dilated vessel diameter (fig. 4, middle panels). The decision as to which of these cause-effect relations mediates the resulting increase in the perfusion reserve cannot be based on theoretical considerations, but will require targeted experimental investigation.

Here, we attribute the evoked vascular reaction to changes in the local O_2 level, not excluding the possibility that other stimuli play a relevant role. During phases of increased activity, local pO_2 and Hb-s O_2 may decrease [44, 107, 108]. Naturally, several other local parameters

change in parallel, including tissue O_2 demand and metabolism, transmural pressure, volume flow and wall shear stress, resulting in marked changes of metabolic and hemodynamic adaptive stimuli.

The vascular activity/hypoxia memory hypothesis is supported by experimental data showing higher levels of resting vascular tone in skeletal muscle compared to more quiescent tissues like the mesentery [98]. Lash and Bohlen [109] studied the adaptations of the spinotrapezius muscle arterioles of rats to 8–10 weeks of treadmill exercise. Exercised rats exhibited a profound increase of functional vasodilation during muscle contraction and an enlargement of the maximal diameter of arterioles compared to sedentary rats, while resting arteriolar inner diameters were essentially identical in both groups. The role of re-

petitive phases of tissue hypoxia for the regulation of vessel tone is also supported by studies on the effects of exercise (14 weeks of treadmill run) in miniature pigs exposed to chronic coronary artery occlusion, which induced collateralization [110, 111]. In this experimental setup, intermittent ischemia occurs during each bout of exercise in collateral-dependent regions. Arterioles (diameter $\sim 150 \mu m$) were isolated from both collateral-dependent and collateral-independent myocardial regions. Chronic occlusion in combination with exercise training produced a profound increase in Ca^{2+} -dependent basal active tone (resting inner vessel diameter was unchanged), while either occlusion or exercise training alone, which it is assumed does not result in hypoxia, had a negligible effect on basal tone.

Fig. 4. Vascular activity/hypoxia memory. Hypothesis for regulation of resting tone level. Left: starting condition. Right: condition after long-term adaptation. Assume there is a vessel with a certain structural diameter and tone, providing adequate blood flow at rest. During repetitive bouts of intense work (first row: blue bars), maximal dilation is elicited but may not be able to prevent hypoxia. An activity/hypoxia-related signal leads to an increase in resting tone (second row: red arrow) or, alternatively, to an increase in structural vessel diameter (third row: blue arrow). For both alternatives, inner vessel diameter at rest has to be maintained to guarantee adequate perfusion at rest. This is achieved by either a compensatory increase in structural (maximally dilated) vessel diameter (blue arrow) or by an increase in resting tone (red arrow), respectively. Dilatory capacity and perfusion reserve provided by resting tone level (green arrows) would therefore also increase from the starting condition, without priming by short hypoxia periods, to conditions after adaptation in response to these hypoxia periods. During the exercise bouts that follow, this increased perfusion reserve is exploited and O_2 levels are better maintained.

The vascular activity/hypoxia memory would act in concert with other adaptive processes observed in response to exercise training, increasing the perfusion reserve of skeletal muscle. Such mechanisms include increased vessel density in the skeletal muscle (see fig. 2, upper panel) and capillary recruitment [112], remodeling of the arterial tree as well as changes in the central and local control of vascular resistance [113].

Characteristics of Metabolic Signaling

Metabolic Signaling by Vasodilators versus Vasoconstrictors

Here we analyze the feedback regulations established by vasodilatory or vasoconstricting metabolic signal substances released from different sources (fig. 5a). We also address effects evoked by the conduction of vasodilatory or vasoconstricting signals to feeding arterioles.

Local Signaling from the Vessel Wall and the Tissue

O₂-dependent metabolic signaling by both vasodilators and vasoconstrictors released from the vessel wall or the parenchymal cells in the tissue establishes a strong and robust local negative-feedback regulation of vessel diameter and blood flow, as described above. For tissue-derived (but not vessel wall-derived) vasodilatory signal substances, this negative feedback may fail when the vessel density is too low [56]. If the distance from a vessel to remote tissue cells exceeds the effective O₂ diffusion dis-

tance, metabolic signals derived from these regions may lead to an increase of the vessel diameter. However, that would not restore adequate tissue oxygenation due to the diffusion-limited O₂ transport decoupling the increase in vessel diameter from the process of generating signals. Such an effect would be prevented if the metabolic signal would exhibit a limited biological life-time and if the effective diffusion distances of the vasodilator and of O₂ were in a similar range.

In contrast, tissue-derived vasoconstrictors are released in proportion to local pO₂ and only from regions that are reached by diffusing O₂, i.e. a perivascular tissue sleeve with a width corresponding to the effective O₂ diffusion distance. Parenchymal cells outside this sleeve thus experiencing anoxia would be silent with respect to vasoconstricting metabolic signaling (but not with respect to required proangiogenic signals), and vasoconstrictors would not evoke an ineffective vessel diameter increase.

In the case of an effective diffusion of metabolic signal substances into the flowing blood, the dilution effect modifies the diameter change evoked by the release rate of a given substance, as described above. For vasodilatory signal substances, an independent negative feedback would be established by the dilution effect supporting and strengthening the O₂-dependent negative feedback. In contrast, for vasoconstricting signal substances, a positive feedback is established that weakens the O₂-dependent negative feedback. In regions of underperfused tissue, a

Fig. 5. Functional settings, vascular control and feedback loops. **a** Feedback loops of metabolic (left) and hemodynamic (right) vascular control. Arrows (blunted ends) indicate stimulating (inhibitory) action. Blue/red lines and -/+ indicate negative/positive feedback while purple lines indicate involvement in both the negative and positive feedback. For better readability, the negative feedback generated by vascular myogenic responses to transmural pressure is not shown. Left: O₂ availability (pO₂ or sO₂) reflecting demand/supply relation determines the production of metabolic signal substances from the vessel wall and the tissue (Wall & Tissue signaling) and from RBCs (RBC signaling), establishing O₂-dependent negative feedback for both vasodilators and vasoconstrictors (large circles). If these substances diffuse to the blood, dilution by the flowing blood evokes an O₂-independent negative feedback for vasodilators but a positive feedback for vasoconstrictors (dilution effect; small circles). In RBC signaling, the quantity of signal producing structures (i.e. RBCs) decreases with decreasing perfusion, causing a positive feedback for vasodilators and a negative feedback for vasoconstrictors (middle circles). Right: hemodynamics. Shear stress-dependent regulation generates a positive feedback because for a given drop in pressure along a vessel, diameter increase (decrease) will evoke an increase (decrease) of flow velocity and lead to an increase (decrease) of shear stress [14].

b Two different physiological settings of vascular control may be considered: steady-state adequate distribution of perfusion in many flow pathways in terminal vascular beds (left) and a substantial increase of perfusion during phases of increased demand, e.g. in muscle exercise (right). Steady state: the colored panel shows flow distribution for steady-state conditions in the microvascular network shown in figure 3. Due to the heterogeneity of microvascular networks, malperfusion can only be avoided if there is a balance between positive- and negative-feedback regulations [17] (lower panel). Increased demand: transient phases of increased O₂ demand call for a strong increase in blood flow (orange line) which requires positive feedback (lower panel) to amplify initial increases of flow. The balance between the negative and positive feedback impacts the potential roles of different metabolic signaling mechanisms in the control of vascular diameter. At rest, metabolic signaling from the vessel wall and the tissue via vasodilators or vasoconstrictors not released into the blood provides the strong negative feedback required to balance the positive feedback generated by vascular reactions to shear stress. During increased demand, the stronger positive feedback components of RBC metabolic signaling and of vessel wall- or tissue-derived vasoconstricting signal substances released into the blood may play a relevant role.

(For figure see next page.)

low blood flow will provide a small distribution volume that promotes an increased vasoconstrictor concentration, competing with the decreased rate of vasoconstrictor production rate due to a decreased pO_2 . Thus, effective vasoconstrictor concentration in the blood may not robustly decrease along with a decreasing pO_2 , as is needed to evoke sufficiently strong diameter increase in underperfused vessels.

RBC Local Signaling

Similar to the signaling from the vessel wall and the tissue, O_2 -dependent metabolic signaling by RBCs via both vasodilators and vasoconstrictors establishes negative feedback signals for the regulation of vessel diameter. However, for a given oxygenation, the rate of signal substances released from RBCs increases with the intravascular volume or flux of RBCs. Microvessels in underper-

fused tissue regions are characterized by both a low RBC content and a low RBC flux. Thus, the total amount of signal substance released and the resulting metabolic stimulus will be low in these vessels. For vasodilators released from RBCs, this evokes a strong positive feedback [56]: the low total amount of vasodilatory signal substance, despite maximal signal production by each RBC in response to low Hb-sO₂, evokes a further decrease in vascular diameter and blood flow, in turn leading to the further reduction of both vessel volume and hematocrit [56, 114]. This positive feedback would amplify any existing heterogeneity of blood flow and hematocrit by vasodilators released from the RBCs. This positive feedback may be converted into a negative feedback only in high-flow vessels and for RBC flux-dependent signaling (decreasing signals with decreasing number of RBCs and vice versa) due to the interdependence of RBC flux and oxygenation that leads to maximum signal strength at a 'threshold RBC flux'. For vasoconstrictors released from RBCs, the same relation between blood flow and quantity of signaling structures would establish an additional negative feedback: in underperfused vessels, low RBC content would cause low production rates and thus weak vasoconstricting effects.

Since RBCs deliver signal substances directly into the blood, the 'dilution effect' would be relevant for RBC signaling and establish an additional negative feedback for vasodilators but a positive feedback for vasoconstrictors, as described above for wall/tissue signaling.

Remote Signaling: Conduction of Metabolic Information to Feeding Vessels

Arterioles supplying many capillaries need to have larger diameters than short arterio-venous connections to prevent the large-scale arterio-venular 'shunting' of blood flow [23]. This is realized by the upstream conductive transfer of metabolic information along the vessel wall [17, 23], with integration of these conducted signals at each branch point. Conducted signals reaching feeding vessels thus correspond to the summed signals from contributing vessels [115]. The stimulus for diameter increase evoked by the conducted signal in a given feeding vessel should increase in proportion to the number of capillaries fed as well as to the degree of O₂ supply/demand mismatch in the tissue supplied by these capillaries.

These requirements for conducted signals are met by vasodilator signaling but not by vasoconstrictor signaling. This can be explained by comparing the signals available in 2 typical cases: a feeding arteriole supplying a large number of capillaries located in a tissue region with a low

pO₂ and a short arterio-venous connection supplying a few capillaries located in a well-oxygenated tissue region. The reactions required in both arterioles would be very different, leading to a large diameter of the feeding arteriole and a small diameter of the short arterio-venous connection. For vasodilators, metabolic signals are maximal for a low pO₂. Thus, the summed conducted signal to the feeding arteriole would be strong (stemming from numerous contributing capillaries with high individual signals) while the conducted signal in the short arterio-venous connection would be weak. These signals would guarantee the required diameter difference. In contrast, in vasoconstrictor signaling, metabolic signals are maximal for a high pO₂. Thus, the same signal level may reach both vessels, as a result of many capillaries each providing a low individual signal (feeding arteriole) or a few capillaries each contributing a strong individual signal (short arterio-venous connection). Consequently, similar diameter reactions would be evoked in both cases by vasoconstrictor signaling, and the resulting relatively too-large diameter of the short arterio-venous connection would lead to blood flow 'shunting' through this flow pathway. A very similar reasoning holds for the downstream convection of metabolic signals which mediates very similar reactions [17, 23].

Metabolic signaling by vasodilators is thus well-suited to adjust perfusion distribution both by vascular reactions to local metabolic conditions and reactions to conducted signals whereas signaling by vasoconstrictors would be adequate only for local responses to metabolic conditions. On the other hand, it might be biologically beneficial for local signaling to reduce the production of the vasoactive mediator in situations of decreased availability of O₂, as is typical for vasoconstrictors, thereby providing a 'fail-safe' mechanism for metabolic signaling. These considerations are based on available information, with more research required to clarify the respective roles of vasodilators and vasoconstrictors which have been shown to simultaneously contribute to the O₂ dependent regulation of vessel diameter [116].

Functional Settings: Steady State and Increased Demand

In vessel diameter adaptation, negative and positive metabolic feedback regulations and hemodynamic feedback regulations compete. With respect to hemodynamic signaling, the vascular myogenic response to transmural pressure may generate a negative feedback (see below), while vascular reactions to flow and shear stress establish a strong positive feedback (fig. 5a, right). For a given pres-

sure drop, an increase in flow leads to an increase in wall shear stress, which in turn leads to an increase in vessel diameter and perfusion. This positive feedback was shown for both the short-term regulation of tone [117] and long-term structural adaptation [118, 119]. For metabolic signaling, the positive and negative feedback characteristics of the different regulatory pathways determine their possible impact on vessel diameter adaptation under resting conditions and conditions of a strong transient increase in demand, e.g. during heavy muscle work (fig. 5b).

Under resting conditions, the central task of metabolic signaling is to provide adequate perfusion distribution within the tissue (fig. 5b, left), despite the fact that the inevitable structural heterogeneity of terminal vascular beds generates heterogeneous perfusion [93]. The positive feedback from wall shear stress signaling which could cause a maldistribution requires balancing by a strong negative feedback from metabolic signaling [23]. The theoretical analyses above suggest that metabolic signaling by vasodilators released from the vessel wall, or its vicinity, possibly supported by the local effects of vasoconstrictors not diluted by the flowing blood, may be best suited to guaranteeing adequate perfusion distribution under resting conditions. This entails robust negative-feedback regulation and transport of vasodilatory information by upstream conduction and downstream convection of metabolic signals [17, 23].

The origin of metabolic signaling under resting conditions was also addressed by experimental studies. In a thorough study on superfused hamster cheek pouch preparations [120], local pO_2 changes were produced by the microapplication of fluid onto the surface of occluded or unoccluded aparenchymal arterioles, or by cannulation and perfusion of arterioles in situ. Perfusion with physiological salt solution equilibrated with a high or low pO_2 did not elicit changes in arteriolar tone, whereas global changes in pO_2 (while the arterioles were perfused by the salt solution), that affected not only the arterioles but also the surrounding parenchyma, produced O_2 -dependent vasomotion. Perfusion of microvascular networks with physiological salt solution retained O_2 sensitivity, eliminating RBCs as the primary sensor of arteriolar O_2 reactivity, suggesting a localization of the O_2 sensor in the tissue [120].

During transient increases of demand (that are very prominent, e.g. in muscle exercise), the main task of diameter adaptation is to amplify bulk perfusion increase (fig. 5b, right). An initial increase of vessel diameter can be mediated by local metabolic signaling. This may be partially counteracted (adding to the negative feedback;

not shown) by myogenic constriction of arterioles distal to the dilation due to the local increase in transmural pressure. However, a positive feedback can be provided by endothelial responses to an increase in wall shear stress, eliciting a further increase in vessel diameter and blood flow [121], which amplifies the initial response and recruits upstream resistance vessels into the reaction [122]. Based on the governing hemodynamic relations, an increase in vessel diameter will lead to an increase in perfusion and wall stress in the typical situation with the driving pressure being maintained (locally, different conditions may prevail [123]). Metabolic vasodilatory signaling by RBCs may provide a further boost, with the perfusion increase and the concomitant hematocrit increase [124] providing an increasing amount of signaling structures (RBCs) traveling through the tissue and a stronger metabolic signal. Thus, while RBC (vasodilatory) signaling is not well-suited to maintaining adequate perfusion distribution to parallel flow pathways under resting conditions, it may play an important role in exercise hyperemia. A corresponding reasoning may apply to vasoconstrictor signaling from the vessel wall or the adjacent tissue, if it is assumed that the effective concentration of the vasoconstricting mediators is affected by local perfusion according to the dilution effect. At a given production rate, an initial increase in diameter and perfusion would lead to increased dilution and reduced concentration of the vasoconstrictor which, in turn, would lead to a further increase in diameter and perfusion, establishing a positive-feedback amplification (see above).

The feedback-based concept of RBC signaling presented here may be able to explain seemingly contradictory experimental in vivo observations. Under resting conditions, replacing blood, and thus RBCs, by saline did not affect vascular responses to changes in pO_2 [20, 120, 125]. In physical exercise, however, blood flow in the skeletal muscle in humans has been shown to primarily respond to a reduction in arterial Hb-s O_2 , and not in pO_2 [126–128], suggesting that a main contribution to O_2 -dependent signaling for blood flow control under these conditions originates from erythrocytes, rather than vascular endothelium, vascular smooth-muscle or skeletal muscle [129].

Summary and Outlook

An integrative analysis of the principal mechanisms and biological reactions involved in metabolic vascular control is used to investigate the role of positive and neg-

ative feedback regulations in achieving the 3 main tasks of terminal vascular networks, i.e. low diffusion distances, a sufficiently homogeneous distribution of perfusion and adequate perfusion reserve to match increases in tissue metabolic demand. This analysis led to 5 hypotheses. (1) The dilution effect can contribute to metabolic vascular regulation and is independent of local oxygenation. (2) A vascular activity/hypoxia memory may control resting vessel tone, and thus perfusion reserve. (3) Vasodilator signaling, but not vasoconstrictor signaling, can prevent shunt perfusion via upstream signal conduction to feeding arterioles. (4) To achieve low-perfusion heterogeneity at a steady state, metabolic signaling from the vessel wall or a perivascular tissue sleeve is optimal. (5) Amplifying perfusion during transient increases of tissue O₂ demand may be supported by RBC-derived vasodilators or vasoconstrictors diffusing into the flowing blood.

Future experimental studies in different tissues are needed to test and further develop these hypotheses. They could include the following investigations: (hypothesis 1) the measurement of the blood concentration of vasodilatory substances produced without or with only a limited relation to O₂ levels as metabolic signal substances as candidates for vascular diameter regulation which may have been overlooked in experimental studies on metabolic vascular regulation, (hypothesis 2) further investigation of vascular gene regulation and expression in microvessels upon repetitive work bouts also addressing the cause-effect relationship between evoked

increases in resting tone and in structural diameter, (hypotheses 3–5) conducting in vivo studies which address the balance between the O₂-dependent release of vasodilators and vasoconstrictors in evoking diameter changes locally and in feeding or draining vessels.

The concepts on metabolic vascular control presented here were derived for a ‘generic’ tissue with ‘average’ properties under physiological conditions. Vessel diameter regulation could be very different in specific ‘real’ microvascular networks and would relate to tissue properties. Further investigation, that combines experiments with theoretical studies, would allow progress in more comprehensively understanding O₂-related metabolic vascular diameter control on the different temporal and spatial scales, at rest and during increased demand as well as the distinctions of this control in different tissues and under different pathophysiological conditions.

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