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REVIEW ARTICLE

The vascular conducted response in cerebral blood flow regulation

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Despite recent advances in our understanding of the molecular and cellular mechanisms behind vascular conducted responses (VCRs) in systemic arterioles, we still know very little about their potential physiological and pathophysiological role in brain penetrating arterioles controlling blood flow to the deeper areas of the brain. The scope of the present review is to present an overview of the conceptual, mechanistic, and physiological role of VCRs in resistance vessels, and to discuss in detail the recent advances in our knowledge of VCRs in brain arterioles controlling cerebral blood flow. We provide a schematic view of the ion channels and intercellular communication pathways necessary for conduction of an electrical and mechanical response in the arteriolar wall, and discuss the local signaling mechanisms and cellular pathway involved in the responses to different local stimuli and in different vascular beds. Physiological modulation of VCRs, which is a rather new finding in this field, is discussed in the light of changes in plasma membrane ion channel conductance as a function of health status or disease. Finally, we discuss the possible role of VCRs in cerebrovascular function and disease as well as suggest future directions for studying VCRs in the cerebral circulation.

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INTRODUCTION

The regulation of blood flow to the brain is under dynamic and precise control to ensure adequate oxygen and nutrient supply as well as to washout metabolic waste products. Regional blood flow control to peripheral tissues is accomplished by neurohormonal mechanisms, and is fine-tuned by local control mechanisms working either through the release of local vasoactive metabolites or mechanical forces such as shear stress or wall stress acting on the endothelium and smooth muscle cell layer of feeding arterioles and precapillary arterioles. The brain, however, encapsulated in a confined space in the skull and protected by the blood–brain barrier, relies almost entirely on local mechanisms to match the demand and supply of energy to the neurons of the brain, a mechanism known as neurovascular coupling.[1] Theoretical work, however, suggests that in arteriolar networks nonlocal mechanisms are necessary for achieving optimal performance with regard to the delivery of oxygen and nutrients.[2,3] It has been speculated that vascular conducted responses (VCRs) could be such a nonlocal mechanism and thereby assist in coordinating and enhancing the effects of changes in local vascular resistances in brain arteriolar networks and thereby contribute to the effective and highly dynamic distribution of blood flow to areas of the brain undergoing large changes in neuronal activity.[4,5]

DEFINITION OF VASCULAR CONDUCTED RESPONSE

A VCR is, by definition, initiated by a local stimulation causing vasodilation or constriction, which rapidly spreads bidirectionally along small blood vessels independent of blood flow or perivascular nerves.[6,7] Most studies have focused on VCRs elicited by local application of agonists to arterioles, but it is clear that local stimulation of either capillaries or small venules may likewise initiate a VCR that spreads upstream into the supplying arterioles.[8,9] Conduction velocity has been estimated to >1 to 3 mm/s in intracellular Ca^{2+} or diameter measurements with limited time-resolution[10–13] and >20 to ~ 45 mm/s in electrophysiological recordings with higher time-resolution.[14,15] For comparison, the velocity of intercellular Ca^{2+} waves spreading along arterioles has been estimated to ~ 0.1 mm/s.[16,17] Thus, conducted vasomotor responses spread with velocities that are 1 to 3 orders of magnitude faster than the spread of intercellular Ca^{2+} waves. The ability of a local vasoactive stimulus to transform into a conducted vasomotor response is dependent on the type of agonist applied, as well as on the vascular cell type targeted by the agonist. For example, it has been shown that a local depolarization imposed on the vascular smooth muscle cell (VSMC) layer in rat renal and mesenteric arterioles conducts with typical length constants of a few hundred micrometers.[12,18,19] Conversely, in skeletal muscle feed arteries and arterioles local application of acetylcholine (ACh), which activates muscarinic receptors on endothelial cells (ECs) and leads to hyperpolarization, conducts with limited decay for up to a couple of millimeters.[15,20,21]

PHYSIOLOGICAL ROLE OF VASCULAR CONDUCTED RESPONSES

The functional role of conducted vasomotor signals within the microcirculation, especially in feed arteries and arterioles, is most

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likely to coordinate changes in resistance and flow in vascular networks, which would enable changes to be manifested more efficiently and rapidly throughout tissues in response to, for example, changes in metabolic need. For example, to meet the metabolic needs associated with an increased neuronal activity, it is necessary to dilate not only the blood vessels within the cortex, but also the larger penetrating and pial arterioles that supply the corresponding cortical area. This necessitates a mechanism that allows local vascular signals from neurons or glial cells to have an effect also on more remote parts of the microcirculation. One such possible mechanism is the VCR.[4] Likewise, in exercising skeletal muscle, an increased metabolic demand leading to dilatation of arterioles deep within the tissue, would benefit from conduction of the dilatation in a retrograde manner to coordinate a simultaneous dilatation of feed arterioles and arteries, resulting in a more efficient and instant supply of oxygen and nutrients to the tissue.[3,22] In the kidney, the tubuloglomerular feedback mechanism, which constricts the afferent arteriole secondary to an increased glomerular filtration rate, is thought to rely in part on the upstream conduction of a depolarization along the glomerular microvasculature.[12,23] In disease states exhibiting an increased microvascular resistance, for example, diabetes or hypertension, it follows that impaired conducted vasodilatation or augmented conducted vasoconstriction could contribute to or even be responsible for the increased arteriolar resistance.

HOW VASCULAR CONDUCTED RESPONSES ARE TYPICALLY MEASURED

The methodological approach depends on whether measurements are performed with isolated arterioles (*ex vivo*) or in an anesthetized animal with some part of its microcirculation exposed for *in vivo*/intravital microscopy measurements. Isolated arterioles are usually mounted between glass pipettes for intraluminal perfusion at physiological pressure. However, very small arterioles may also be studied without pressure and flow, being positioned at the bottom of a recording chamber using suction micropipettes or bioadhesive proteins.[24] Measurements of local and conducted diameters require a light microscope equipped with a digital or video camera for recording of the experiments. The local responses are elicited by placing the tip of a micropipette (tip 1 to 5 μm) adjacent to an arteriole for application of agonists such as norepinephrine (NE), phenylephrine (PE), ACh, or KCl, etc. Often a microiontophoresis device is used to deliver agonists, which ensures a tight control of agonist delivery. For delivery of sufficient quantities of KCl, it is necessary to use a pressure microejection device. Alternatively, a local response may be elicited by direct electrical stimulation via a micropipette whereby the vessel is exposed to a train of unipolar pulses causing perturbations of the membrane potential.[25,26] The experiments are usually performed by initially recording the local response a few times, then moving the objective to an upstream location for recording of the conducted (remote) response to repetitive local stimulation (see Figure 1). Alternatively, the stimulation site is moved to different downstream sites while the conducted responses are observed consecutively at a fixed upstream site. The distance to the upstream location is usually between 500 to 2,000 μm depending on the type of vessel and agonist applied.

The change in EC and VSMC intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) have also been measured during a VCR. Here, local and conducted measurements were performed using either ratiometric (Fura-2, Fura-PE3) or single-wavelength excitation (Fluo-3) of Ca^{2+} indicators using epifluorescence microscopy.[11,13,27–29] For simultaneous recording of Ca^{2+} signals at multiple sites along an arteriole during a VCR, an objective with low magnification ($\times 20$) and high numerical aperture and a sensitive CCD camera for capturing a sufficient amount of fluorescence has been used.[13,18] Conducted vasoconstriction is associated with an increase in

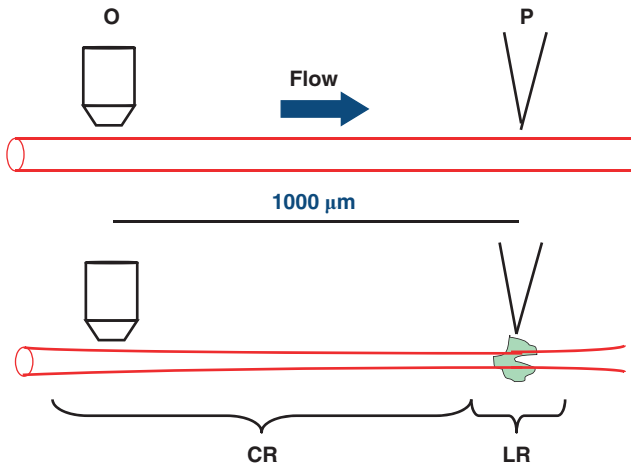

Figure 1. Experimental approach for measurement of conducted vasomotor responses in arterioles (here depicted as local and conducted vasoconstriction). A micropipette (P) tip for delivery of agonist (green shade) is placed adjacent to an arteriole. It is important to make sure that the agonist is carried away from the vessel with the superfusate flow. At 1,000 μm in the upstream direction, the arteriole diameter is recorded through a microscope objective. The magnitude of the conducted response (CR) is seen to decay with distance from the local response (LR). See text for further details.

$[Ca^{2+}]_i$ in both the VSMC and the EC, and the change in $[Ca^{2+}]_i$ showed an attenuation with distance from the local stimulation site that paralleled the degree of vasoconstriction.[13,18,27,29] The wide-field recordings of $[Ca^{2+}]_i$ can be used as a measure of the conducted signal, and therefore provides detailed information on the strength of the conducted signal along the vessel. Such data have enabled a detailed mathematical description of the conduction process by nonlinear curve-fitting and computer simulations.[18]

The electrical intercellular communication underlying the conduction of vasomotor responses along the vascular wall has been measured using sharp microelectrodes inserted in either VSMC or EC at various locations along the arteriole.[27,30–33] Usually investigators impale only one cell at the time and stimulate at various locations along the vessel successively, ending up with a number of V_m measurements along the conduction pathway obtained at different time points. A few studies have also obtained V_m recordings in arterioles at dual sites, making it possible to record conducted responses in VSMCs and ECs simultaneously, or to inject current at the local site and record V_m deflections at the conducted site.[15,30] This technique has provided crucial insight into which cell type(s) is involved in initiation and rapid spreading of the VCRs along the vessel wall.

Intravital microscopy for recording of VCRs in arterioles from intact, anesthetized animals has been performed using the hamster cheek pouch preparation, the rat mesenteric preparation and the mouse cremaster muscle preparation. These studies have mainly reported diameter measurements, but importantly Ca^{2+} dynamics in ECs *in vivo* were recently reported using a transgenic mouse expressing a GCamP2 Ca^{2+} sensor under the control of a Cx40 promotor found only in ECs of the vasculature and Purkinje fibers of the heart.[16] Finally, sharp microelectrode measurements of V_m in VSMCs and ECs during VCRs in the above-mentioned *in vivo* models are routinely performed in only a few laboratories.[10,32,34,35]

MOLECULAR AND CELLULAR MECHANISMS INVOLVED IN CONDUCTION OF VASOMOTOR SIGNALS

There is consensus in the literature that conducted vasodilatation is preceded by spreading of a hyperpolarization between the cells

in the vascular wall, and that conducted vasoconstriction is initiated by a local depolarization conducted intercellularly to distant sites. However, the cell type(s) involved in these processes is still under debate, and seems to depend on the nature of the local stimulus and the cell type stimulated. As an example, local application of ACh onto an arteriole activates muscarinic receptors on the endothelium at the local site, which leads to $G_{\alpha/q}$ activation, diacylglycerol, and inositol triphosphate (IP_3) production causing Ca^{2+} store release and capacitative Ca^{2+} entry through receptor- and storage-operated cation channels in the ECs.[36,37] This activates endothelial small and intermediate conductance Ca^{2+}-activated K^+ channels ($K_{Ca}2.3$ and $K_{Ca}3.1$), which leads to a hyperpolarization of ECs. The ECs are interconnected by gap junctions and the local endothelial hyperpolarization therefore spreads longitudinally from EC to EC along the length of the vessel.[20,38–41] Subsequently, the hyperpolarization spreads from the ECs into the VSMCs through gap junctions in the myoendothelial projections.[42–45] As ECs are 30 to 140 μm long and oriented along the longitudinal axis of the vessel, each EC may establish contact with as many as 20 VSMCs.[46,47] The hyperpolarizing current from ECs to VSMCs results in a reduction in the number of open voltage-gated (L-type) Ca^{2+} channels in the VSMCs, a fall in VSMC $[Ca^{2+}]_i$ and vasodilatation. As the hyperpolarization spreads along the endothelium, more and more VSMCs are hyperpolarized and eventually relaxed.

In the case of a vasoconstrictor, such as NE or PE applied locally onto arterioles and activating $G_{\alpha/q}$-coupled receptors in VSMCs, both monophasic conducted vasoconstriction as well as biphasic local vasoconstriction followed by a conducted vasodilatation has been observed depending on the vascular bed under study.[25,48,49] In the first case, a local depolarization caused by activation of receptor-operated cation channels and/or PKC-mediated inhibition of Ca^{2+}- and/or voltage-activated K^+ channels in VSMC, is conducted along the vessel through gap junctions coupling VSMCs with VSMCs. In addition, the depolarization may spread into the underlying endothelium, and can be conducted through this cellular pathway along the vessel wall. As the depolarization spreads into distant VSMCs, L-type channels are activated and the concomitant Ca^{2+} entry and rise in $[Ca^{2+}]_i$ leads to conducted vasoconstriction. In the latter case where a transient local vasoconstriction is followed by a secondary conducted vasodilatation, evidence has shown that a local $G_{\alpha/q}$-mediated IP_3 release and Ca^{2+} increase in VSMC may spread via myoendothelial gap junctions into adjacent ECs to increase their local $[IP_3]$ and $[Ca^{2+}]_i$ to activate endothelial Ca^{2+}-activated K^+ channels thereby causing a secondary conducted hyperpolarization and vasodilatation.[48–51] Application of a local high KCl concentration has also been widely used as a tool to induce conducted depolarization and vasoconstriction. This leads to a conducted vasoconstriction of rather limited amplitude, which is thought to rely primarily on intercellular communication via the VSMC layer. Interestingly, the tendency of local vasoconstrictor application acting on VSMCs to induce conducted vasoconstriction with limited amplitude compared with agonists acting on ECs, have been explained by a higher dissipation of current through gap junctions and ion channels for VSMC-initiated responses. Thus, with a sufficiently strong local depolarization of VSMCs to overcome current dissipation, the depolarization can spread into adjacent ECs and be conducted with limited decay along this pathway as well.[52]

As noted, the KCl-induced conducted signal is smaller in amplitude compared with the vasomotor signals induced by local vasodilator or vasoconstrictor hormones, which are often conducted without significant decay within the distances of 1 to 2 mm usually investigated in this type of study. This has led to the hypothesis that a regenerative mechanism exist in the vascular wall, which would account for propagation of a hyper- or depolarization over long distances in the microcirculation.[15,26,35,53] In hamster retractor muscle feed arteries, it was shown that inward-rectifier (K_{IR}) K^+ channels possess the inherent biophysical properties necessary to facilitate the conducted hyperpolarization and vasodilatation to local ACh application. Using a range of *in vitro* methods and computational modeling, it was shown that the negative-slope conductance of K_{IR} channels during hyperpolarization of VSMCs would augment the initial hyperpolarization as it conducts through VSMCs along the vascular wall.[54] Thus, this was the first concrete molecular evidence of a regenerative mechanism.

Previous studies suggested that voltage-gated Na^+ channels may be expressed in the vascular wall, either in ECs[53] or in sensory nerve terminals adjacent to arteriolar VSMCs[55] and that activation of these channels might contribute to the regenerative conduction process. This topic is still not completely resolved, but recent studies did not find a role for TTX-sensitive[13,25,29] or TTX-insensitive Na^+ channels[18] in conducted depolarization in rat renal or mesenteric arterioles.

Recently, a new hypothesis argues against the requirement of a regenerative mechanism for nondecaying conducted vasodilatation. This model, which gained support from experimental evidence in mouse cremaster arterioles *in vivo*, proposes that the conducted hyperpolarization is more negative than the range of membrane potentials at which L-type channel window currents occur, thus causing the vasodilatation to be maximal over a long segment of the arteriole, while the conducted hyperpolarization in fact decays electrotonically.[34] Thus, only at large distances from the local site is the hyperpolarization small enough to allow a limited Ca^{2+} entry through L-type channels, which would tend to decay the conducted vasodilatation. It will be interesting to see this model investigated in more vascular beds and using virtual arteriolar models incorporating the crucial ion channels and intercellular resistances known to affect conduction.

PHYSIOLOGICAL MODULATION OF VASCULAR CONDUCTED RESPONSES

An interesting question is whether VCRs are subject to physiological or pathophysiological modulation in the sense that the extent of the conduction can be modified by physiological factors, for example, hormones or nervous activity. The extent or spread of a VCR can be expressed by its length constant λ. The length constant is the distance from the site of stimulation where the response has decayed to $\sim$63% of the initial value.[7,56] The intercellular electrical circuit of an arteriole consists of an inner conduction pathway with resistance R_j determined by the combined gap junctional and cytoplasmic resistances, and an outer semipermeable leak pathway consisting of the plasma membrane with the resistance R_m. In analogy with electrotonic conduction in axons, the length constant λ in arterioles depends on the ratio between the resistance of the plasma membrane and the resistance of the intercellular compartment: $\lambda = \sqrt{(R_m/R_j)}$. This implies that the conducted responses can be regulated by modulating either the gap junctional resistance or the resistance of the plasma membrane. The latter is primarily determined by the activity of the K^+ channels present in the cell membrane.[57,58]

The gap junctional resistance depends on the number of gap junctional channels, the single channel conductance and the open probability.[59] Several factors, like changes in gene activity, intracellular trafficking of connexins, $[Ca^{2+}]_i$, intracellular pH, and posttranslational modifications of connexins, for example, phosphorylation, are able to modify one or more of these three parameters, and, thus, modulate gap junctional resistance.[59] The connexins found in the vascular wall are primarily Cx37, Cx40, Cx43, and Cx45.[59] Cx40 appears to be the dominant connexin, and it is found primarily in the ECs. In accordance with the central role of gap junctions, Cx40 knockout mice has impaired conducted vasodilation,[20] and nonspecific inhibitors of gap junctions like carbenoxolone or palmitoleic acid completely abolish the VCR.[13,60]

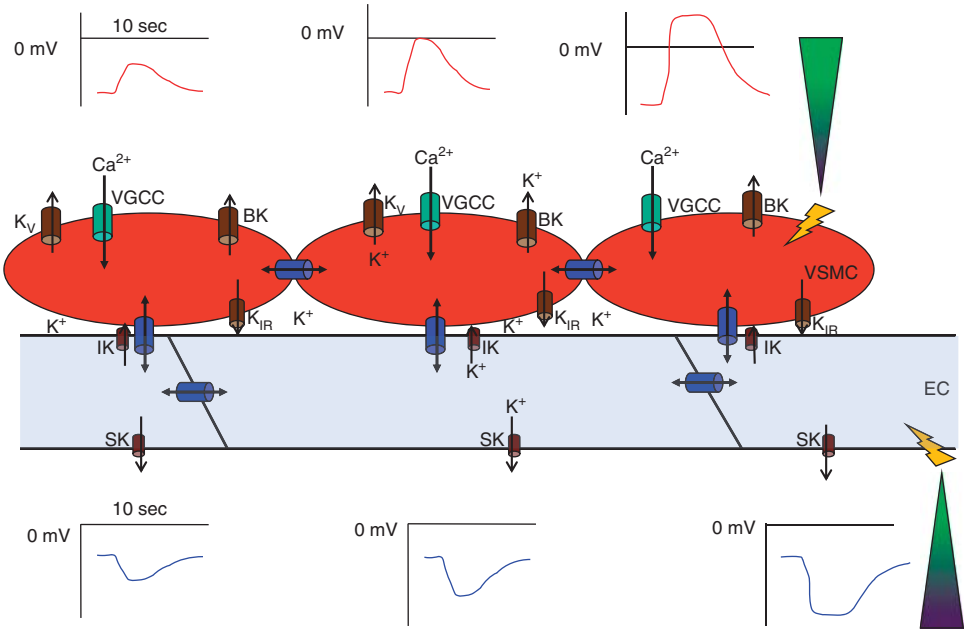

Figure 2. Conduction mechanism involved in vascular conducted responses (VCRs). The schematic shows coupled endothelial cells (ECs) and vascular smooth muscle cells (VSMCs) in the arteriolar wall, equipped with ion channels and gap junctions necessary to sustain conduction through the intercellular compartment(s) along the vessel. On the right, two pipette tips (green color) show delivery of a depolarizing (upper panel, VSMC) and hyperpolarizing (lower panel, EC) current to initiate conduction along the vessel. Both the depolarization (red curve) and hyperpolarization (blue curve) is seen to decay with distance from local stimulus. Blue barrels depict gap junctions coupling EC–EC, and VSMC–VSMC, as well as myoendothelial coupling (EC–VSMC). Green barrels depict voltage-gated Ca^{2+} channels (VGCC) whose activity convert the de- or hyperpolarizations into an increased or decreased Ca^{2+} influx into VSMC. Brown barrels depict various K^+ channels in EC and VSMC, whose function is to modify the electrical responses as they are conducted along the EC and VSMC pathways. See text for further explanations. BK, big conductance Ca^{2+}-activated K^+ channels; IK, intermediate conductance Ca^{2+}-activated K^+ channels; SK, small conductance Ca^{2+}-activated K^+ channels; K_V, voltage-gated K^+ channels; K_{IR}, inward-rectifier K^+ channels. The duration bar is arbitrarily set to 10 seconds in the plot of V_m versus distance. The ECs and VSMCs are not drawn to scale.

Despite the central role of gap junctions in VCR, there are at present no experimental data to show that modifications of gap junctional resistance plays a role in physiological or pathophysiological modulations of VCRs.

In a recent study, the hypothesis was tested that an increase of R_m caused by inhibition of plasma membrane K^+ channels would lead to augmented conducted vasomotor responses in rat mesenteric terminal arterioles. Using an experimental and computational approach, it was demonstrated that BK_{Ca} and K_V channels limit conducted vasoconstriction in intact rat mesenteric arterioles by effectively dissipating current out of the VSMCs and limiting the transfer of current to other cells in the wall via gap junctions.[18] As both BK_{Ca} and K_V channels are depolarization-activated, it is an interesting question whether conducted hyperpolarization could be modified by plasma membrane K^+ channel activity. In rat mesenteric small artery, simultaneous inhibition of BK_{Ca} and K_V channels using a cocktail of pharmacological drugs, lead to augmentation of conducted hyperpolarization and vasodilatation to local ACh and isoproterenol application.[61] Presumably, these blockers eliminate the dissipation of hyperpolarizing current along the arteriole; however, it is somewhat unexpected to see an effect of inhibiting these voltage-gated channels as hyperpolarization could be expected to completely close BK_{Ca} and K_V channels. Recently, however, nonvoltage-gated endothelial SK_{Ca} and IK_{Ca} channels were also shown to limit the conducted hyperpolarization to local ACh in an isolated endothelial tube preparation from mouse epigastric arteries,[62] demonstrating that the major K^+ conductances in both ECs and VSMCs can play a role in modifying the conducted vasomotor responses by constituting a regulated leak pathway in the vascular wall as the conducted signal is passing through the cells. This dissipation of current effectively limits the conduction and would therefore serve as a means of physiological regulation of conduction and perhaps explain the difference in λ between responses obtained in different animal models with altered ion channel expression as a consequence of a treatment or disease.

Aging is associated with a reduction in BK_{Ca} channel expression and function in rat coronary and skeletal muscle arteries,[63,64] and this might alter conducted vasomotor responses in arterioles from aged individuals. In hypertensive animals, cerebral artery BK_{Ca} channels are upregulated,[65] whereas in diabetic mice and rats the β_1-subunit of the BK_{Ca} channel is downregulated, leading to impaired function of the channel.[66,67] Thus, it can be expected that the changes in BK_{Ca} channel expression and function modifies the length constant of VCRs in these diseases. Having defined a key role of K^+ channels for modulation of VCRs, it can be predicted that intracellular regulators of K^+ channel activity, such as PKC, PKA, 20-HETE, epoxyeicosatrienoic acid (EETs), prostaglandins, and PIP_2 would be capable of regulating VCRs.

Interestingly, such effects may explain the modulation of VCRs that have been observed under certain conditions. Conducted vasoconstriction to local depolarization or NE application in mesenteric terminal arterioles was augmented by systemic Ang II infusion and abolished by the Ang II receptor antagonist losartan in anesthetized rats.[68] In isolated rat mesenteric terminal arterioles preconstricted with NE and neuropeptide Y, the λ for conducted vasoconstriction to local depolarization was increased in a similar manner.[18] We suggest that the action of systemic or topical administration of $G_{\alpha/q}$-coupled receptor agonists to modulate the VCRs may be explained by PIP_2 depletion or PKC-mediated inhibition of vascular BK_{Ca} and K_V channels. In contrast to the above effects of agonists, sympathetic nerve activation in skeletal muscle arterioles was previously noted to inhibit conducted vasodilatation to local ACh application. This effect was mediated via α_1- and

α_2-adrenergic receptors, and it was hypothesized that NE released from arteriolar varicosities caused a decrease in R_m by opening depolarizing ion channels in VSMCs, which would increase the dissipation of conducted hyperpolarizing current along the vessel.[69]

The length and branching of the vessel(s) under study will also influence the VCRs. Short arteriolar segments with electrically sealed ends tend to have larger remote responses, and thus larger λ values, because of the smaller total plasma membrane area available for dissipative currents. On the other hand, increasing vessel length can be expected to cause increased dissipation of current into the intercellular and extracellular compartments. When estimating λ, it is therefore important to utilize an equation that incorporates the method of reflection that takes into account the variable degree of dissipation as a function of vessel length.[56,70] Branching of the vessels, as in an intact microcirculatory network, would also effectively dissipate the conducted vasomotor signals due to significant current dissipation into the vascular cells along the side branches.[31]

Figure 2 summarizes the molecular and cellular mechanisms involved in conduction and modulation of VCRs in arterioles. Homocellular coupling of ECs and VSMCs is achieved through gap junctions between neighboring cells, and heterocellular coupling occurs by gap junctions in myoendothelial projections passing through the internal elastic lamina. Voltage-dependent Ca^{2+} channels are the effectors linking the conducted electrical signals with the appropriate relaxation or contraction of VSMCs. Small and intermediate conductance Ca^{2+}-activated K^+ channels are important for initialization of the conducted hyperpolarization to a local rise in EC $[Ca^{2+}]_i$. In some vascular beds, the negative-slope conductance of inward-rectifier K_{IR} channels augment hyperpolarizations as they conduct through the VSMC pathway. Finally, several types of K^+ channels in EC (SK_{Ca}, IK_{Ca}) and VSMC (BK_{Ca}, K_V) limit intercellular conduction of electrical signals due to charge dissipation across the cell membrane.

VASCULAR CONDUCTED RESPONSES IN THE CEREBRAL MICROCIRCULATION

The most intensively studied conducted vasomotor responses in the cerebral circulation are those initiated by local ATP, ADP, or adenosine application onto rat cerebral penetrating arterioles (passive diameter $<100\,\mu$m) isolated from middle cerebral arteries. Here, local adenosine application elicited both local and conducted vasodilatations,[71,72] which conducted rapidly in a decaying manner.[71] ATP and ADP caused initial local vasoconstriction followed by a secondary local vasodilatation, which was conducted rapidly and in a decaying manner to remote sites.[71–73] The local constriction to ATP was inhibited by low concentration of pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid (3 μM) and α,β-methylene ATP (1 μM), showing that P2X-receptors are involved.[73] The local secondary dilatation was reduced by impairment of endothelial function using air emboli, while the local constriction was enhanced by this procedure.[73] The local dilatation was also sensitive to blockers of NOS (nitric oxide synthase) and Cytochrome P450 (CYP450), as well as to inhibition of endothelial IK_{Ca} by luminal application of TRAM-34, and to abluminal application of iberiotoxin to inhibit smooth muscle BK_{Ca} channels. Upon local ATP application a transient depolarization followed by a hyperpolarization preceded the local vasomotor responses.[73] Taken together, these data indicate that local ATP activates smooth muscle P2X receptors causing local depolarization and vasoconstriction. At the same time, local ATP activates endothelial P2Y receptors, leading to a local rise in EC $[Ca^{2+}]_i$, which activates NOS, PLA_2, and CYP450 enzymes. The local EC Ca^{2+} increase will cause hyperpolarization and secondary local dilatation by activating endothelial IK_{Ca} channels directly,[36,58] and indirectly activating VSMC BK_{Ca} channels through a PLA_2/CYP450/EET-dependent mechanism.[74,75] The conducted dilatations to local ATP were dependent on an intact endothelium and were preceded by conducted hyperpolarization,[73] consistent with electrotonic conduction and electromechanical coupling of the conducted vasomotor responses as observed in other systemic arterioles.[15,27,32] Nitric oxide synthase or cyclooxygenase inhibition did not affect the conducted vasodilatation. CYP450 inhibition, on the other hand, strongly attenuated both local and conducted dilatations to ATP.[73] However, the CYP450 inhibitor was applied to the bath and not via pipette to the local or remote sites, so it is difficult to conclude whether EETs play a role in the local response only, or in the conduction process *per se*. As EETs are known to activate Ca^{2+}-activated K^+ channels, it cannot be excluded that EET activation along arterioles could theoretically contribute to conducted hyperpolarization. However, EETs are usually released in response to receptor activation and/or local Ca^{2+} increases and it is therefore difficult to imagine how EETs could be activated on a time scale fast enough to account for the spreading electrotonic hyperpolarization underlying the conducted vasodilatation in arterioles. The data presented by Dietrich *et al*[73] suggest that the conducted vasodilatations to ATP are initiated by local hyperpolarizations and conducted electrotonically in a decaying manner via intercellular coupling of ECs and spread via myoendothelial junctions to the VSMC layer to cause remote vasodilatations.

In another study[76] on isolated cerebral penetrating arterioles (passive diameter $\sim 55\,\mu$m), local elevation of [KCl] from 3 to 5 mM produced a minor local constriction followed by robust dilatation, and the dilatation conducted rapidly >1 mm with minimal decay. The local initial constriction was most likely caused by an initial Nernstian depolarization induced by the change in E_K in VSMCs. The local secondary dilatation was blocked by ouabain but not by $BaCl_2$ applied luminally or abluminally, indicating that activation of the Na^+/K^+-ATPase but not K_{IR} channels mediate the local hyperpolarization and dilatation to a local $[K^+]$ increase from 3 to 5 mM.[76] The local hyperpolarization seems to be conducted through the endothelial pathway, since the conducted dilatation to local KCl was significantly reduced after passing air emboli through arterioles. The conducted dilatations were reduced, but not abolished, by abluminal $BaCl_2$, whereas there were no effects of blocking K_V, K_{Ca}, or K_{ATP} channels using a combination of 4-aminopyridine, tetraethylammonium, and glibenclamide.[76] These data suggest that K_{IR} channels function to amplify the conducted hyperpolarizations, most likely due to their inherent negative-slope conductance as suggested for skeletal muscle arterioles.[54] Bath application of ouabain blocked the conducted dilatations to the same extent as the local dilatations, but ouabain was not applied locally, so it cannot be determined whether the Na^+/K^+-ATPase contributes to the conduction process *per se*. It is interesting that the conducted dilatations only decayed slightly with distance. This suggests that cerebral penetrating arterioles may have: (1) a very well-coupled endothelial layer, (2) expression of K_{IR} channels that may act as amplifiers of conducted hyperpolarizations, (3) a hyperpolarized voltage threshold beyond which dilatations are maximal, and (4) no major dissipative ion currents in EC or VSMC during conducted hyperpolarizations. If these requirements are met, the conducted dilatations could theoretically spread almost without attenuation over large distances in unbranched arterioles.

In rat penetrating arterioles (passive diameter 55 to 70 μm) isolated from pial arteries, local ATP application elicited local vasoconstriction followed by vasodilatation, as well as a conducted vasodilatation.[5] In pial arterioles, prostaglandin F (PGF)2α elicited local vasoconstriction and simultaneous conducted vasodilatation. Conducted dilations to ATP and PGF2α were interpreted to be mediated via an endothelium-dependent mechanism.[5] In pial arterioles, local adenosine caused local vasodilatation, but did not consistently produce conducted dilatation.

In an *in vivo* study of pial arterioles (15 to 40 μm) in halothane anesthetized rats equipped with an open cranial window,

stimulation of parallel fibers in the cerebellar cortex produced both strong local vasodilatation, presumably caused by a local production of glutamate leading to local NO and adenosine release, as well as an upstream remote vasodilatation in larger feeding arterioles outside of the activated cortex area.[4] However, in this latter study, it could not be excluded that part of the upstream vasodilation was due to flow-mediated vasodilation.

Taken together, pial and cerebral penetrating arterioles do possess powerful conducted vasomotor responses that are conducted electrotonically via an endothelial pathway. The latter study[4] demonstrates the important concept that local increases in neuronal activity are coupled with upstream dilatation of arterioles at sites outside the stimulated area, indicating an important role for conducted vasomotor responses in neurovascular coupling.

PERSPECTIVES FOR STUDYING CEREBRAL VASCULAR CONDUCTED RESPONSES

The ultimate goal in any physiological and pathophysiological study of the microcirculation in the brain is to gain knowledge of the *in vivo* function of the brain. Therefore, in addition to studying isolated cerebral arterioles, the study of VCRs *in vivo* would be highly desirable. However, the anatomy of the brain and its blood supply offers considerable obstacles in pursuing *in vivo* studies of the important penetrating arterioles. These are not readily visible on the brain surface, but branch downwards into the brain. It seems probable that such studies would be advanced by employment of sophisticated imaging techniques enabling researchers to record responses deep within brain tissues, coupled with spatially well-defined focal stimulation of brain arterioles. Such methods are already available, for example, multiphoton microscopy of brain arterioles[77,78] and localized uncaging of Ca^{2+} or IP_3 in a single or few cells.[79–81] As gap junctions, voltage-gated Ca^{2+} channels and endothelial-dependent responses are known to be sensitive to anesthetic concentrations of propofol, thiopental, halothane, isoflurane, sevoflurane, and other general anesthetic agents[82–87] it is of paramount importance to validate the method of anesthesia carefully in such *in vivo* studies.

POTENTIAL ROLE OF VASCULAR CONDUCTED RESPONSES IN CEREBROVASCULAR DISEASE

Subarachnoid hemorrhage is associated with regional cerebral hypoperfusion that is treatment-resistant and may cause prolonged episodes of regional brain ischemia. It has been speculated that the associated hypoperfusion to subarachnoid hemorrhage could be caused by either augmentation of conducted vasoconstriction or impairment of conducted vasodilatation in penetrating cerebral arterioles. By mimicking the effects of locally released oxyhemoglobin during subarachnoid hemorrhage, Kajita *et al*[72] showed that the conducted vasodilatations to local ATP, ADP, and adenosine application were markedly reduced in isolated cerebral penetrating arterioles exposed to oxyhemoglobin, and in parallel the conducted vasoconstriction to PGF2α was increased.

The VCRs are most likely also important for reperfusion following brain ischemia. A recent study in rats investigated the conducted vasodilatation to local ATP or adenosine application after experimental middle cerebral artery occlusion at 1 and 24 hours reperfusion, respectively. It was demonstrated that conducted vasodilatation to ATP and adenosine was augmented after prolonged reperfusion, only. Thus, it is possible that regional hypoperfusion and ischemia in the brain is compensated for by augmentation of conducted dilatation without concurrent changes in conducted vasoconstriction.[71]

Cortical spreading depression (CSD) is associated with changes in the diameter of arterioles on the cortical surface, as well as changes in blood flow distribution in the cortex. A recent study reported that a wave of vascular dilatation is running ahead of the leading edge of CSDs induced by local depolarization of the cortex in anesthetized rats and mice equipped with cranial windows.[88] The spreading dilatation was independent of underlying cortical or parenchymal activity and consistently followed the pattern of arteriolar networks on the cortical surface and was never observed to traverse cortical areas independent of arterioles. Furthermore, the rate of spreading dilatations was about twice the rate observed for CSDs (4 mm/min versus 2 mm/min) and this lead the authors to speculate that changes in the local parenchymal chemical environment, such as astrocytic Ca^{2+} waves, could trigger an electrical event that might initiate conducted vasodilatation along parenchymal arterioles.[88] However, it must be pointed out that the conduction velocity of conducted vasomotor responses is several orders of magnitude faster than the spreading dilatations observed in the latter study. Although this could be influenced by the choice of model or anesthetic agent, we would argue that conducted hyperpolarizations along the endothelial pathway leading to remote dilatations, cannot explain the phenomenon of CSDs. Much slower intercellular Ca^{2+} waves have been described in ECs in culture,[89] in isolated vessels,[17] and *in vivo*.[16] The intercellular Ca^{2+} waves are dependent on the PLC pathway, IP_3 diffusion, Ca^{2+}-induced Ca^{2+} release, and gap junctions.[90] It remains to be investigated whether intercellular Ca^{2+} waves in brain blood vessels are involved in CSD.

CONCLUSIONS AND PERSPECTIVES

The conduction of vasomotor responses along the arteriolar wall depends on intercellular communication of an initial local de- or hyperpolarization along the electrically coupled cells of the vascular wall, and on the degree of current dissipation across the plasma membrane through several types of ion channels. Although the local stimuli to initiate VCRs in cerebral arterioles might not be the same as in other systemic arterioles, the mechanisms of conduction do not seem to be different. The role of VCRs in regulating cerebral perfusion in health and disease deserves more attention due to their potential involvement in neurovascular coupling and in conditions under which brain ischemia might occur. The development of methods for studying VCRs in cerebral arterioles *in vivo* is needed to advance our understanding of their role in brain blood flow control in health and disease.

DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

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