1. Introduction

Glaucoma and diabetic retinopathy are consistently the leading causes of blindness in Japan. The progression of these retinal diseases may be associated with structural and/or functional abnormalities in the retinal circulation (1–4). The retinal vasculature lacks autonomic innervation (5). Therefore, circulating hormones and local factors released from endothelial cells and surrounding retinal tissues might play a key role in regulating retinal blood flow (5) (Fig. 1). The ability to normalize the retinal circulation by correcting or maintaining neuronal and glial function would prevent or delay the progression of retinal diseases.

In this review, we will address the following 3 issues: i) neurovascular interactions established in the retina, ii) pathological alterations of these neurovascular interactions in diabetic retinopathy and glaucoma, and iii) rationales for novel therapeutic approaches to correct and/or reestablish the interactions among multiple cell types including neuronal, glial, and vascular cells.

2. Neurovascular interactions in the retina

2.1. Neurovascular coupling in the retina

Neuronal activity and local blood flow in the central nervous system are tightly coupled, known as functional hyperemia (6). This response provides additional oxygen and nutrients to match the increased metabolic demands of active neurons. Initially, the homeostatic response was thought to be mediated by signals from neurons to vascular cells. However, recent evidence suggests that glial cells also contribute to neurovascular coupling in the brain (7, 8). Regarding the retina, taking advantage of this tissue, such as direct visual accessibility of blood vessels and light sensitivity of the neural circuitry, both the existence of a tight adjustment between retinal blood flow and neuronal activity and the contribution of glial cells to the adjustment have been demonstrated (9).
2.2. A mechanistic understanding of neurovascular coupling

Several mechanisms have been proposed to explain the increase in retinal blood flow response induced by flicker-light stimulation (increase in neuronal activity). For example, as is the case for neuronal activity–dependent vasodilation in the brain (10), nitric oxide (NO) appears to be an important contributor because inhibitors of NO synthase (NOS) attenuated flicker light–induced vasodilation in the optic nerve head and in the retina (11). Synaptically released glutamate, which is a major excitatory neurotransmitter in the retina as well as in other regions of the central nervous system, acts on N-methyl-D-aspartate (NMDA) receptors in neurons to raise intracellular Ca\(^{2+}\), which in turn activates neuronal NOS. The released NO relaxes vascular smooth muscle cells by increasing intracellular cGMP. In addition to the vasodilator effect, NO modulates glia-to-vessel signaling (12). The addition of NO donors attenuates the vasodilation induced by light stimulation as well as glial cell activation (12). In retinal arteries, the presence of perivascular nitregic nerves has been demonstrated (13). Therefore, NO derived from the nitregic nerves could also contribute to the regulation of retinal circulation.

The transmitters released from neurons can induce the elevation of intracellular Ca\(^{2+}\) in glial cells (14), leading to the activation of phospholipase A\(^2\) and the production of arachidonic acid (8). Arachidonic acid is metabolized into a number of vasoactive compounds, including prostaglandins and epoxyeicosatrienoic acids (EETs), which dilate blood vessels, and 20-hydroxyeicosatetraenoic acid (20-HETE), which constricts blood vessels (7, 12). Thus, the activation of glial cells results in vasodilation or vasoconstriction under different conditions (12). Glutamate appears to be the principal transmitter from neuronal cells to glial cells in the brain (15), whereas in the retina Müller cells and astrocytes that are major glial cells are activated by ATP rather than glutamate released from neurons (16). Experiments using purinergic antagonists have shown that signals from neurons to astrocytes are mediated by ATP and glial purinergic P2Y receptors (16).

Increases in neuronal activity in the brain can facilitate the opening of large conductance Ca\(^{2+}\)-activated K\(^+\) (BK\(_{Ca}\)) channels on astrocyte endfeet mediated by increases in Ca\(^{2+}\) (17, 18). The opening of BK\(_{Ca}\) channels facilitates the efflux of K\(^+\) from astrocyte endfeet, which can in turn dilate cerebral vessels. This K\(^+\)-related
mechanism seems to contribute to functional hyperemia because the blockade of BKCa reduces vasodilation (18). However, in the retina, K⁺ efflux from glial cells is unlikely to contribute significantly to functional hyperemia (19).

Delaey and Van de Voorde demonstrated the presence of perivascular cells that control the vascular tone of isolated retinal arterioles. These effects are mediated by diffusible chemical messengers (20). Further studies revealed that NMDA can act on perivascular cells to dilate retinal arterioles (21). This finding suggests that retinal cells expressing NMDA, such as retinal ganglion cells and amacrine cells, control vascular tone. However, the observed vasodilator effect of NMDA is likely mediated by adenosine that is synthesized by hydrolysis of ATP, which is released after the stimulation of NMDA receptors on perivascular cells. Although the role of glial cells in this phenomenon remains unclear, these results also suggest that metabolic changes influence vascular tone in the retina.

2.3. Role of retinal ganglion cells in the formation and maintenance of a vascular network

In the mouse, the retinal vasculature begins to develop after birth, extends throughout most of the retina by the 7th day, and is fully developed by approximately 20 days. However, in mice lacking retinal ganglion cells (RGCs), no retinal vascular network is ever established (22). RGCs express the GPR91 receptor for the Krebs cycle intermediate succinate, which accumulates in hypoxic areas of the retina, and knockdown of the receptor on RGCs substantially diminished pathologic neovascularization in an oxygen-induced retinopathy model (22). Thus, RGCs appear to play an important role in both physiologic and pathologic retinal angiogenesis.

The contribution of RGCs to the maintenance of retinal blood vessels was established through experimental models of retinal regeneration induced by ischemia–reperfusion or an intravitreal injection of excessive dose of NMDA (23, 24). In these models, retinal damage, such as retinal ganglion cell apoptosis and/or thinning of the inner retina, precedes subsequent capillary degeneration (23, 25). These findings strongly suggest that RGCs play a role in maintaining the normal structure and function of retinal blood vessels.

2.4. Role of circulating catecholamines

Retinal blood vessels lack sympathetic innervation (5) but express a- and β-adrenoceptors (26, 27). Therefore, autonomic nervous system activation may alter retinal vascular tone by increasing the release of catecholamines from the adrenal medulla. Our studies on rats showed that: i) intravenously injected 1,1-dimethyl-4-phenylpi-

perazinium (DMPP, a ganglionic nicotinic receptor agonist) dilates retinal blood vessels (28) and ii) adrenaline induces the vasodilation of retinal blood vessels by stimulating β2- and β3-adrenoceptors (29), while noradrenaline induces the constriction by stimulating α1A- and α1D-adrenoceptors (30). These findings suggest that activation of the autonomic nervous system enhances the release of adrenaline from the adrenal medulla and thereby triggers retinal vasodilation through the stimulation of β2/β3-adrenoceptors. On the other hand, the exogenous administration of noradrenaline does not alter retinal blood flow in humans (31). The authors concluded that even high levels of circulating noradrenaline have little impact on the regulation of retinal blood flow. However, there is still limited information about the role of circulating catecholamines in the control of retinal blood flow.

3. Neurovascular interactions in diabetic retinas

Diabetic retinopathy has a complex pathology that affects both neuronal and vascular elements of the retina (4). Abnormalities in the retinal vascular system, including basement membrane thickening, pericyte loss, and terminal arteriole occlusion, usually serve as early signs of the development of diabetic retinopathy (4). In addition to such morphological changes, the flicker light–induced vasodilation of retinal blood vessels is also diminished in diabetic patients (32, 33). This diminished response was detected before the clinical appearance of diabetic retinopathy. Similarly, in streptozotocin-induced type 1 diabetic model rats, despite a marked reduction in light-induced vasodilation, no effect on retinal thickness or neuronal cell number was documented. The study did report early glial reactivity and an up-regulation of inducible NOS (iNOS) (34). On the other hand, there are also reports suggesting that neurodegenerative events may precede these vascular changes (35). Thus, the cause-and-effect relationship between neural cell death and vascular dysfunction during diabetic retinopathy remains unclear.

The mechanisms responsible for the diminished flicker light–induced dilation observed in diabetic retinas are still not fully understood. However, the pathologic changes in retinal neuronal, glial, and vascular cells could contribute to reduction in flicker light–induced vasodilation and may consequently deprive retinal neurons of oxygen and nutrients. This chain of events would exacerbate diabetic retinopathy.
4. Neurovascular interactions in glaucoma

Glaucoma is the most common optic nerve head neuropathy and is associated with a loss of RGCs and visual field damage. The elevation of intraocular pressure (IOP) is a primary risk factor for glaucoma; however, the disease progresses in a large portion of patients despite therapeutic intervention to lower IOP, and a subset of glaucoma patients exhibit optic neuropathy despite normal IOP (36). Therefore, other factors such as an abnormal regulation of ocular blood flow might be associated with the pathogenesis of this disease (1, 37).

Diminished flicker light–induced retinal vasodilation is observed in glaucoma patients as well (38, 39). This phenomenon may be, at least in part, the result of an abnormal response in retinal blood vessels due to endothelial dysfunction and an imbalance between endothelial-derived contracting and relaxing factors (3). Reduced neural activity and altered glial cell function (40, 41) may also contribute to the diminished vasodilation. A decrease in the number of capillaries in the optic nerve head and atrophy of the peripapillary capillaries supplying the retinal nerve fiber layer have been reported as well (42).

5. Targeting neurovascular interactions for the treatment of retinal diseases

Studies of neurovascular coupling in animal models or patients with diabetic retinopathy or glaucoma clarify the pathogeneses of these retinal diseases and provide the opportunity to develop drugs to optimize neuroglia-vessel interactions. In this section, rationales for novel therapeutic approaches based on the interaction between retinal neuronal cells and their blood supply will be discussed.

5.1. GABA<sub>C</sub>-receptor agonists

Qian and Ripps proposed an interesting hypothesis: decreasing the activity of inner retinal neurons will reduce the metabolic demands of the retinal cells and thereby diminish ischemic/hypoxic stress in the retina (43). γ-Aminobutyric acid (GABA) is the main inhibitory neurotransmitter in both the central nervous system and the retina (44). Among 3 major classes of GABA receptors, the GABA<sub>C</sub> receptor is of special interest in the context of this hypothesis for several reasons: for example, i) the GABA<sub>C</sub> receptors are highly expressed on the axon terminals of retinal bipolar cells where they contact inner retinal neurons, ii) GABA<sub>C</sub> receptors mediate sustained responses with little sign of desensitization, iii) GABA<sub>C</sub> receptors exhibit higher agonist sensitivity and therefore the desired inhibition of inner retinal neurons will be achieved by relatively low doses of drugs, and so on (43). Their studies on experimental animals revealed that a GABA<sub>C</sub> agonist affords a form of pharmacological intervention that is capable of reestablishing balance between the blood supply and neuronal activity, which can in turn relieve hypoxia in the damaged retina.

5.2. iNOS inhibitors

Increased iNOS expression is commonly observed in diabetic and glaucomatous retinas (45, 46). Overproduction of NO could induce retinal ganglion cell apoptosis (47). High levels of NO in retinal tissue do not appear to compromise vascular responsiveness, but inhibit the glial release of dilatory agents, which disrupts the coupling between neuronal activity and vasodilation, resulting in reduced vasodilation and enhanced vasoconstriction (12). Therefore, it is likely that the inhibition of iNOS and reduced retinal NO levels facilitate normal neurovascular signaling (34, 48).

5.3. Endothelin-1 (ET-1)-receptor antagonists

Endothelin, the most potent vasoactive peptide known to date, has been suggested to play a role in the pathogenesis of open-angle glaucoma. Plasma and aqueous humor concentrations of ET-1 have been shown to be elevated in patients with ocular hypertension as well as in the corresponding animal models (49 – 51). Chronic local administration of ET-1 to the optic nerve head reduces optic nerve head blood flow and is associated with a loss of retinal ganglion cells (52). In addition to these vascular effects, ET-1 is implicated in pathological changes in the retina such as neuronal cell apoptosis, microvascular basement membrane thickening, and gliosis (53). Furthermore, ET-1 contributes to the regulation of IOP through effects on the trabecular meshwork, which is the main route for the outflow of aqueous humor from the eye. Because endothelins could contribute to the detrimental neuronal, vascular, and glial effects seen in glaucoma, drugs that block their action should be considered as potential tools in the pharmacological treatment of glaucoma (54, 55).

5.4. Angiotensin II antagonists

Experimental and clinical evidence has established the presence of a local renin–angiotensin system (RAS) in the retina (56). The components of the RAS have been identified in vascular, glial, and neuronal cells and contribute to the pathogenesis of several retinal diseases, such as diabetic retinopathy and retinopathy of prematurity, i.e., enhancement of retinal neovascularization, inflammation, oxidative stress, and neuronal and glial
dysfunction. These abnormalities can be attenuated by treatment with angiotensin-converting enzyme inhibitors and angiotensin II type 1 (AT1)-receptor antagonists (57). Therefore, compounds acting on the RAS may have the potential to correct the neuro–glia–vessel interaction and could ultimately be used for the treatment of diabetic retinopathy. They may also lower IOP and exert a neuroprotective effect in patients with glaucoma.

6. Conclusion

Increasing evidence demonstrates that components of the neurovascular unit act as an intricate network to maintain a homeostatic neuronal microenvironment in the retina. In retinal disease, the interruption of cell-to-cell communication could represent an additional risk factor for diabetic retinopathy and glaucoma. Therefore, future efforts to develop drugs for the treatment of retinal disease must aim to correct and/or reestablish the interactions among the cells that make up the neurovascular unit.

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