Analysis of a potential new model for neurovascular coupling in retina and its relation to the retinal relaxing factor.

Since the first observations that retinal blood flow is higher in flickering light than in constant light, it is believed that there is a coupling of metabolism and blood flow in the eye. Adaptation of local arterial smooth muscle tone to neuronal activity (neurovascular coupling) is extensively studied in brain. Much less is known about the neurovascular coupling in retinal circulation. In the cerebrovascular circulation astrocytes can relay signals from neurons to vascular cells and in this way function as intermediaries between neurons and cerebral blood vessels to adjust blood flow to neuronal activity. Potential astrocyte-derived vasoactive mediators of cerebrovascular responses include for instance epoxyeicosatrienoic acids, prostaglandins, potassium and CO.

About one decade ago our research group discovered that retinal tissue continuously releases an as yet unidentified retinal relaxing factor (RRF)\(^1\). This was established in experiments on isolated bovine retinal arteries mounted for isometric tension recording. When a piece of retinal tissue was brought in close contact with a precontracted bovine retinal artery, this elicited a very strong relaxation that disappeared after removal of the retinal tissue. It was also found that contractility of bovine retinal arteries with adherent retinal tissue was much weaker than that of retinal arteries without retinal tissue. The fact that RRF-induced relaxation was much more pronounced in hypoxic conditions indicated that this factor may be involved in neurovascular coupling in the retina. This research was in part supported by FRO grants offered to Christophe Delaey and Koen Boussery.

During our recent research on RRF we observed that electrical field stimulation (EFS) of isolated and precontracted bovine retinal artery does not affect tone. This is in line with the absence of autonomic regulation of retinal circulation. However, when a retinal artery is surrounded by adherent retinal tissue, EFS (train duration 20sec; frequency 8Hz; pulse duration 5msec; 80V) elicits a rapid and reversible vasorelaxation (about 30 \%). The aim of the present project is to characterize this vasorelaxing effect, which may lead to a better understanding of neurovascular coupling in retina.

Experiments will be performed using isolated retinal arteries with adherent retinal tissue mounted for isometric tension measurements in an oxygenated (5 \% CO\(_2\) in O\(_2\)) Krebs-Ringer bicarbonate solution at 37°C (pH= 7.4). After precontraction of the preparations with PGF\(_{2\alpha}\) (30 \(\mu\)M) EFS will be applied by a Grass stimulator via two parallel platinum electrodes on each side of the retinal artery.

In a first series of experiments the frequency dependency of EFS will be investigated. In addition the influence of EFS will be investigated on non-precontracted preparations. This can reveal potential vasocontractile mediators that may simultaneously be released during EFS and may play a confounding role as modulators of the relaxation effect seen during EFS on precontracted preparations.

In a next series of experiments it will be investigated whether EFS-induced retinal relaxation depends on activation of neuronal activity using tetrodotoxin. In addition experiments will be done using \(\omega\)-conotoxin MVIIIC, which inhibits calcium entry through presynaptic voltage-gated calcium channels (N-, P- and Q-type). This entry is an essential step in neurotransmitter release.
We will also investigate whether astrocytes are involved in the neurovascular coupling in retina, using L-2-alpha aminoacidic acid, a selective astrocyte toxin. Astrocytes are believed to be accurate sensors of neuronal activity. Astrocytes respond to the synaptic release of glutamate with oscillations in the intracellular calcium concentration. Glutamate-mediated \([\text{Ca}^{2+}]\), elevations in astrocytes trigger the release of vasoactive compounds \(^2\). Therefore we will also investigate the influence of glutamate receptor antagonists that inhibit neuronal activity dependent \([\text{Ca}^{2+}]\), elevations in astrocytes, but not in neurons.

In another series of experiments we will address the question what vasoactive molecule(s) (released from glial cells and/or neurons) is involved in the EFS-induced relaxing response. One possibility is that under EFS more of the continuously liberated RRF is released by retinal cells. As the RRF response is inhibited in the presence of 120 mM of K\(^+\) \(^1\) we will investigate the influence of 120 mM of K\(^+\) on EFS-induced relaxations.

However, besides RRF many other neurotransmitters and glial cell-derived molecules can be considered as potential mediators of EFS-induced relaxation. The potential release of acetylcholine from cholinergic neurons, one of the most widely studied neurotransmitters in the retina \(^3\), will be assessed using atropine. To investigate the involvement of NO released from nitrergic neurons, NO-synthase inhibitor nitro-L-arginine will be used. Given that activation of astrocytes triggers the release of prostaglandins \(^2,^4\) and epoxyeicoatrienoic acids \(^3\), we will investigate the influence of cyclooxygenase (COX) inhibitor indomethacin and the cytochrome P450 epoxygenase inhibitor miconazole on the EFS-induced relaxing response. Extensive evidence suggest that CO is a glutamate-induced, diffusible messenger used by astrocytes to signal for arteriole dilation by activation of smooth muscle cell Ca\(^{2+}\)-sensitive K\(^+\) (K\(_{\text{Ca}}\)) channels \(^6\). Therefore, we will investigate the involvement of CO, using a K\(_{\text{Ca}}\) channel blocker such as paxilline, which blocks CO-induced vasodilation. Another possibility is the release of K\(^+\) by astrocytic K\(_{\text{Ca}}\) channels into the perivascular space to activate smooth muscle cell inward rectifier K\(^+\) (K\(_{\text{ir}}\)) channels and cause vasodilation \(^7\). Therefore, we will investigate the influence of blocking K\(_{\text{Ca}}\) and K\(_{\text{ir}}\) channels on the EFS-induced vasorelaxing response.

It is hoped that the result of these experiments would gain a better insight into the link between retinal metabolism and blood flow.

References:


