PERSPECTIVE

Neuroprotection of the inner retina: Müller cells and lactate

Müller cells: The neglected neighbor: Müller cells constitute the majority of retinal glial cells and offer more alternating functions than any other cell of the retina. Uniquely, Müller cells cover the complete thickness of the retina, and their roles therefore differ correspondingly to the retinal segment in which they are located. In the inner retina, Müller cells are crucial in taking up toxic molecules, such as excessive glutamate from the synapses between bipolar cells and retinal ganglion cells (RGCs), thereby preventing glutamate-induced excitoxic RGC death (Bringmann et al., 2009; Skytt et al., 2016; Toft-Kehler et al., 2016; Vohra et al., 2017) (**Figure 1**). Additionally, Müller cells are crucial in maintaining ion balances and have also been suggested to secrete essential neuroprotective factors as well as to buffer energy sources to the neighboring cells (Bringmann et al., 2006, 2009). Despite being over looked for decades Müller cells have now been proven essential in overall retinal maintenance, and increasing attention and acknowledgement has been attributed to their mere presence and function.

In general, Müller cells contain elevated amounts of mitochondria and are known to be extremely resistant to various forms of pathogenic conditions, such as hypoxia, hypoglycemia and oxidative stress (Toft-Kehler et al., 2016; Vohra et al., 2017), indicating that the Müller cells' defense towards toxic stress might be defined by their energy metabolism and increased adenosine triphosphate (ATP) turnover. However, in the case of excessive stress or multiple toxic factors, the defense mechanisms may diminish and result in dysfunctional Müller cells (Toft-Kehler et al., 2016; Vohra et al., 2017). As a compensatory self defensive mechanism, the Müller cells might take up alternate energy substrates, such as glutamate, to accommodate the need for a greater energy consumption (Skytt et al., 2016; Toft-Kehler et al., 2016, 2017) (**Figure 1**).

Recently, Müller cells were proposed to take up lactate from the outer retina and merely transfer lactate to inner retinal neurons as an alternate energy substrate (Hurley et al., 2015). However, we challenge this assumption by arguing that Müller cells may in fact metabolize lactate (Vohra et al., 2018), which during compromised glucose availability may be essential in upholding their protection of inner retinal neurons.

Although human peripheral blood lactate levels lie between 1–2 mM, physiological levels of retinal lactate extend up to 5–50 mM depending on the species. Certainly, these high levels of lactate must play a role in maintaining retinal function and survival (Kolko et al., 2016).

In line with this, our previous studies have shown increased uptake of radioactively marked glutamate by the human Müller cell line, MIO-M1 in response to 10 mM of extracellular L-lactate (Vohra et al., 2018), thus verifying a boosted Müller cell function in the presence of lactate.

Moreover, our recent paper highlighted numerous novel features of lactate uptake in Müller cells such as sustained glycogen storage and increased survival, indicating a shift in scientific reasoning towards lactate being more than merely a metabolic waste product.

The present perspective article aims to highlight the importance of Müller cell energy metabolism with special attention to lactate-linked neuroprotection in the inner retina.

Lactate: More than a metabolic waste product in the retina: Lactate is produced by the end-step of glycolysis, which marks the first metabolic pathway of retinal energy metabolism. In most of the human body, the breakdown of glucose *via* glycolysis provides 2 ATP and 2 pyruvate molecules, and lactate is therefore mainly produced during low oxygen availability, such as ischemia and excessive exercise. However, retinal cells have been shown to produce lactate even during sufficient oxygen supply, a phenomenon known as the Warburg effect. More interestingly, the over all rate of retinal lactate production exceeds the over all rate of retinal pyruvate production. Proposedly, this may yield a fast ATP turnover, suitable for the energy demanding retinal cells *e.g.*, Müller cells, whilst oxidative phosphorylation of the pyruvate-derived, Acetyl coenzyme A, is a time consuming, tedious process.

After being formed, lactate is either released from Müller cells to the extracellular surroundings (Vohra et al., 2017) or metabolized internally as an energy substrate (Vohra et al., 2018). Interestingly, metabolism of lactate in Müller cells has been shown to sustain stores of glycogen, potentially sparring glucose as glycogen for events in which glucose supply is limited (Vohra et al., 2018). Moreover, over all ATP levels have also been shown to remain stable, even during low energy supply (absence of lactate and glucose), which could be explained from both glycogen degradation and/or metabolism of amino acids. In line with this, Müller cells have additionally been shown to increase their uptake of glutamate, which is then used as an alternate energy substrate (Skytt et al., 2016; Toft-Kehler et al., 2016; Vohra et al., 2017). Likewise, lactate may potentially also be used as an energy substrate when secreted to neurons.

The secreted lactate may, however, also facilitate other functions besides being an active metabolic substrate. Thus, we speculate whether lactate actually possess signaling properties, which similar to hormones is tightly regulated through feed back systems. In line with this, we recently revealed a high abundance of the lactate-receptor G protein-coupled receptor 81 (GPR81) in the retina (Kolko et al., 2016). GPR81 activation has been linked to neuronal activity and energy substrate availability in neuronal tissue (Lauritzen et al., 2014; Morland et al., 2015). GPR81 is located by the dendritic spines close to the synapses in the brain (Morland et al., 2015). The molecular mechanism involved in GPR81 activation constitute decreased levels of cyclic adenosine monophosphate (cAMP), followed by constrained protein kinase A (PKA) activation and subsequently reduced intracellular calcium levels (Lauritzen et al., 2014). Consequently, such cascade reaction will lead to an even more negative membrane potential, thereby

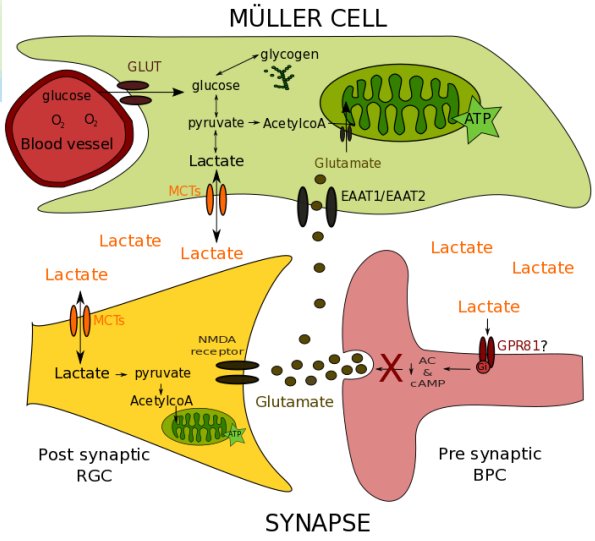

Figure 1 Effects of energy metabolism and lactate-mediated responses in inner retinal synapses.
Müller cells receive energy substrates from either blood glucose or from extracellular lactate within the retina. Both energy substrates are able to enter the tricarboxylic acid cycle (TCA) cycle as Acetyl coenzyme A (AcetylcoA) formed by pyruvate, resulting in production of adenosine triphosphate (ATP) in the mitochondria. Lactate, however, is also shuttled to surrounding nerve cells, such as BPCs and RGCs, where it may be used as an energy substrate. Moreover, lactate may also function as a signaling molecule by G protein-coupled receptor 81 (GPR81) activation, which results in a Gi-mediated pathway inhibition of adenylate cyclase (AC) followed by reduced cyclic adenosine monophosphate (cAMP) levels. In turn, this will result in diminished neurotransmitter release. Additionally, Müller cells take up extracellular glutamate to prevent excitotoxic damage of the RGC. GLUT: Glucose transporter; MCTs: monocarboxylate transporters; EAAT: excitatory amino acid transporter; NMDA: N-methyl-D-asparate; RGC: retinal ganglion cell; BPC: bipolar cell.

hindering the release of neurotransmitters. In other words, neurotransmission will potentially be compromised during the presence of lactate or activated GPR81 receptors. Thus, we believe that lactate serves a regulatory purpose in neuronal signaling, which may prevent excessive release of glutamate from bipolar cells to RGC (**Figure 1**). Lactate-mediated protection against glutamate toxicity might also occur in a synergistic manner targeting both neurons (bipolar cells) and glial cells, since Müller cells also enhance their uptake of glutamate in the presence of lactate.

This corresponds nicely with current knowledge of lactate regulation and function retrieved from studies in the brain, which suggest a potential feedback system of lactate in regards to decreased glutamate toxicity, increased cellular energy metabolism and ameliorated neuronal survival. Hence, these findings may be extrapolated to the retina, ultimately leading to increased neuroprotection of RGCs in response to lactate (**Figure 1**), which could be applicable knowledge in the treatment of various neurodegenerative retinal conditions.

Targeting Müller cell metabolism and signaling of lactate in the treatment of glaucoma: The most devastating consequence of retinal neurodegeneration is blindness. In this context, glaucoma is the most frequent cause of irreversible blindness worldwide due to a progressive loss of the RGCs. Due to the age-related nature of glaucoma, statistical calculations estimate twice as many patients with glaucoma globally in 2040 (Tham et al., 2014). Generally, glaucoma is linked to increased intraocular pressure (IOP), but is classified by the progressive loss of the RGCs and their axons. Current treatments focus solely on the IOP-lowering aspect. Although IOP-lowering drugs decrease the rate of glaucomatous progression in most patients, the treatments offer no possible visual rescue and 15% patients with diagnosed glaucoma will eventually go blind. Thus, the need for sufficient therapeutics is inevitable. Previous pharmaceuticals attempting to rescue RGC have, however, been unsuccessful.

This might possible be due to the multifactorial pathogenesis of glaucoma. Hence, several factors acting either on neuronal cell bodies or their axons are believed to cause RGCs death. Generally, elevated IOP and vascular dysregulation are hypothesized to cause the initial insult by obstruction of the axoplasmic flow of the RGC and altered optic nerve microcirculation at the level of lamina cribrosa. Initially, compromised blood flow might result in decreased energy and oxygen supply, which may prevent the RGCs from surviving. Since Müller cells are located between the blood supply and the RGCs, these cells might actually be the first target of insult. Consequently, dysfunctional Müller cells may lead to reduced protection of RGC (**Figure 1**).

Secondary insults of RGC include excitotoxic damage caused by glutamate released from injured neurons and oxidative damage caused by increased production of reactive oxygen species (ROS) in response to the varying oxygen supply (Toft-Kehler et al., 2016). ROS is often accumulated in the cellular organelles and may therefore result in dysfunction of the mitochondria, which then restricts cellular energy metabolism. Since Müller cells and their mitochondria might be affected prior to retinal neurons, they are still capable of producing lactate through glycolysis and secrete it to neighbouring neurons. Thus, lactate might be secreted and used as an energy substrate by the RGC, which may initially still have intact mitochondria.

Regardless of the primary and secondary factors of glaucomatous damage, the end result involves dysfunction and loss of RGCs leading to irreversible visual loss.

Due to the complexity of the pathophysiology behind glaucomatous neurodegeneration, we believe that previous treatment strategies to rescue RGCs have failed, since initiation of treatment was done too late in the disease course, *i.e.*, at a point where the RGC were already dead.

Instead, we propose that future treatments should intervene at an earlier time point in the disease progression prior to advanced RGC loss. In our opinion intervention at such an earlier time point could ensure the functional maintenance of the neighbouring Müller cells.

As highlighted previously, Müller cells are pivotal in preventing RGC death. Hence, the inner retinal disorder, glaucoma, may admissibly be avoided by enhancing Müller cell function. This concept has also been deecribed as reactive Müller cell gliosis, which is a cellular attempt to protect tissue from further damage and promote repair and remodeling (Bringmann et al., 2006). Although, gliosis of Müller cells has also been linked to cytotoxic effects of retinal neurons, such as release of several pro-inflammatory molecules and nitrosative stress, the over all initial effects are predominantly neuroprotective (Bringmann et al., 2006). In a recent study, the presence of extracellular lactate was shown to boost Müller cell survival and function, theoretically resulting in lactate-mediated neuroprotection of RGCs. Thus, lactate-linked pharmaceuticals may be of great future interest, ultimately leading to novel therapies in the cure of retinal neurodegeneration, such as glaucoma.

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