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Ischemia is defined as an inadequacy of blood flow to tissue. Ischemia can deprive tissue of oxygen and metabolic substrates and it can also prevent the removal of waste products. If the ischemia is maintained over enough time the tissue will lose its homeostasis and eventually die causing an infarct. Retinal ischemia occurs when the blood supply to the retina does not meet the metabolic needs that are required to sustain the retina. This can lead to retinal damage and severe vision loss. Ischemia is caused by occluded blood vessels.

The retinal blood supply originates in the ophthalmic artery and branches into the different sections of the retina. The external retina, which contains the cell bodies of the photoreceptors, is provided with nutrients by the choroid blood vessels. The inner layer and ganglion layer, which contains the ganglion bipolar and amacrine cell bodies, is nourished by the central retinal artery (CRA) (Brown, 1991).

Complete retinal ischemia occurs when the opthalamic artery is occluded. Major causes of retinal ischemia are central retinal artery occlusion (CRAO), branch retinal artery occlusion (BRAO), central retinal vein occlusion (CRVO), and branch retinal vein occlusion (BRVO). CRAO is caused by either a thrombosis of the CRA or an embolisation of an artherosclerotic internal carotid artery (Brown, 1991). Ischemia caused by the CRAO leads to an infarction and loss of function of all the inner layers of the retina while the outer layers of the retina including a portion of the inner nuclear layer remain intact (Kincaid et al, 1998).

The CRA branches off into several different branches in order to perfuse the inner layer of the retina. BRAO occurs when one or some of these vessels are occluded. BRAO has the same pathology and risk factors of CRAO but affects more of a specific area of the inner retina depending on which branch is occluded (Brown, 1991).

Venous occlusive diseases are more common clinically than arterial occlusions. The risk factors for CRVO are similar to those of CRAO but also include an elevated intraocular pressure (IOP) or an underlying hematological disease (Mitchell et al., 1996).

Ionotropic Receptors

Ionotropic receptors are a group of transmembrane ion channels that are opened or closed in response to the binding of a chemical messenger. NMDA (N-methyl-D-aspartic acid), AMPA (α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate), and kainite receptors are ionotropic receptors in neurons that are activated by glutamate which is a main neuron neurotransmitter (Digledine et al 1999). NMDA receptors are responsible for regulating the influx of calcium and other ions in the cell. Normally the channel is blocked by Mg^{2+} ions (Eby and Eby, 2006). Under normal circumstances a nearby AMPA receptor is activated during resting polarization by glutamate, which starts depolarization. This depolarization spreads to the NMDA receptor and it releases the Mg^{2+} ions. Once the NMDA receptor is open glutamate will activate the receptor, which causes an influx of Ca^{2+} and Na^{+}. Ca^{2+} is used by the cell

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to activate metabolic pathways and reactions in the cell.

Kainate receptors are not as well understood as other receptors but its conductance is very similar to that of AMPA receptors.

Metabotropic Glutamate Receptors

Metabotropic receptors differ from ionotropic receptors, like NMDA and AMPA, in that instead of activating an integral ion channel they activate secondary messengers usually G-proteins, which activate ion channels on the plasma membrane. To date eight distinct subtypes of metabotropic glutamate receptors (mGluRs) have been identified and separated into three groups based on function and sequence homology (Thoreson and Witkovsky, 1999). Group 1 mGluRs (mGlu1 and mGlu5) are usually located postsynaptically and enhance the cell's excitability by mobilizing intracellular Ca2+ and activating protein kinase C, which leads to several signal transduction pathways.

Group II (mGlu2 and mGlu3) and Group III (mGlu4 and mGlu6-8) are typically found presynaptically. The transduction process of these receptors involve a negative coupling with adenylate cyclase and their activation causes a reduction in glutamate release which lowers synaptic excitability (Nicoletti et al, 1996).

Ischemic Cascade

On the cellular level death from ischemic attack is brought about by many factors. The start of the cascade comes from the lack of oxygen and glucose supply. This leads to decreased rates of glycolysis and oxidative phosphorylation, which causes levels of ATP to fall (Lipton, 1999).

The main impact of the decrease of ATP in the neuron is the inhibition of the Na^+/K^+-ATPase transporter, which is a transporting enzyme on the cell membrane. The main function of this enzyme is to restore the resting potential of the neuron after it has been hyperpolarized from the release of excess potassium. When the neuron becomes hyperpolarized the Na^+/K^+-ATPase actively transports sodium ions out of the cell and potassium ions inside the cell to restore the neuron to its resting potential so that it can depolarize and make an action potential. Once this enzyme is inhibited the voltage dependent Mg^{2+} on the NMDA (N-methyl-D-aspartic acid) receptors becomes loose which allows for an excess uptake of calcium by the cell (Zeevalk and Nicklas, 1992). Ionotropic AMPA (α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate)/kainite type glutamate receptors also become more activated which leads to an influx of ions and osmotically obligated water which can lead to cell lysis. The inhibition of the Na^+/K^+-ATPase is the cause of the hyper activation of these receptors.

These receptors are activated by glutamate, which is an extracellular neurotransmitter in the brain. During ischemia, through some proposed mechanisms, which will be elaborated later, there is an increase in extracellular glutamate levels, which in turn activate the now active receptors during reperfusion, which leads to cell death as will be explained later on in this paper. This rise in glutamate levels has been documented in several studies (Neal et al, 1994; Shimada et al, 1993).

The early mechanism proposed for the increase in extracellular glutamate levels was that after the opening of the Ca^{2+} channels there would be an increase in exocytotic neurotransmitter release through normal metabolic pathways. This is because an increase in Ca^{2+} in a cell will cause an increase in cell metabolism. However, due to the depletion of ATP in the cell, this mechanism cannot really account the very high levels of glutamate because of the lack of energy available for this process to occur (Nishazawa, 2001).

The main candidate for an alternative mechanism is the reverse transport of glutamate transporter proteins. The transport of glutamate into the cell in order to prevent excessive depolarization is not driven by ATP but rather by the Na^+ gradient inside the cell and the concentration of K^+ and pH-changing anions out of the cell (Barbour et al 1998). When ischemia occurs and Na^+/K^+-ATPase begins to reduce in activity, the Na^+ gradient is decreased and the K^+ accumulates in the extracellular space, which may reverse glutamate uptake (Attwell et al, 1993). This mechanism was proposed when it was noticed that there was an increase in extracellular glutamate in anoxic conditions (David et al, 1988). Other studies have shown that glutamate levels are increased when there is either an increase in extracellular K^+ on intracellular Na^+ (Barbour et al, 1991).

Normally glutamate is taken up by glia and converted by glutamine synthetase into glutamine and returned back into the neural soma (Thoreson and Witkovsky, 1999). Ischemia lowers the ATP levels in glia and causes a decrease in the conversion of glutamate. This causes an increase in the glutamate/glutamine ratio, which may also lead to extracellular glutamate (Oliver et al, 1990).

Once the cell constantly becomes depolarized excess calcium enters the cell and sets off many cascades that lead to cell death. Among the most critical are free radical production and nitric oxide (NO) synthesis, activation of phospholipase A_2, DNA cleavage, activation of proteases, and subsequent damage to cytoskeleton. One of the main influences of the high calcium level is the formation of superoxide radicals (Bonne et al, 1998). Calcium activates a dependent protease calpain, which converts xanthine dehydrogenase to xanthine oxidase. Upon reperfusion, this enzyme converts hypoxanthine to uric acid, which results in the release of superoxide radicals (Chan, 1996). One of the major ways that superoxide damages the cell is that it attacks unsaturated fatty acids which lead to lipid perioxidation of membranes (Doly et al, 1984). This will result in loss of membrane fluidity, cell swelling, oedema, and feed forward production of more oxygen-derived radicals. There are many other pathways that cause cell damage that stem from the increase in calcium ions, which can lead to the activation of procaspases which leads to apoptosis.

The following figure illustrates the different steps in retinal ischemia.

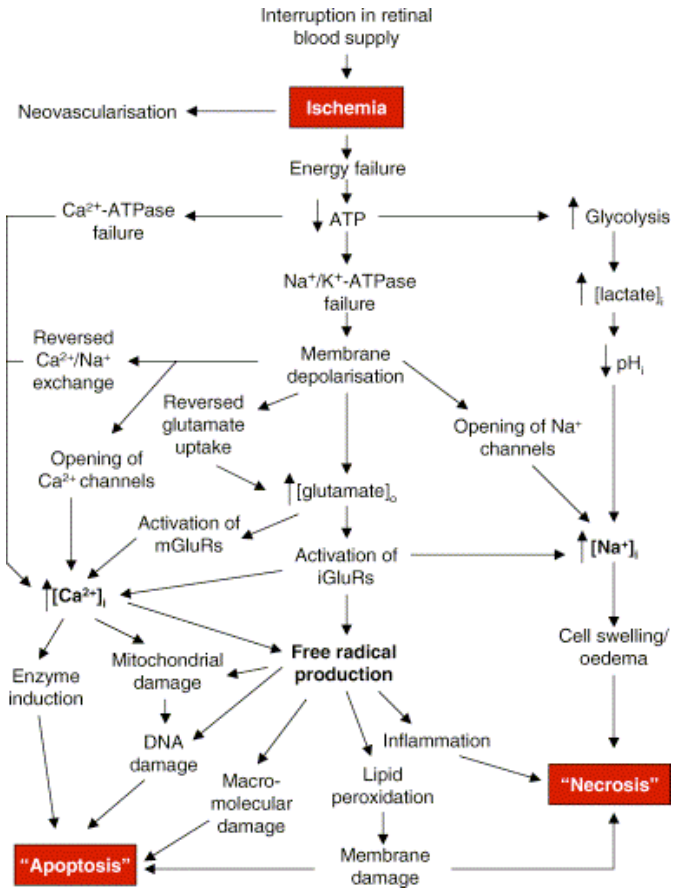

Fig. 1. Summary of hypothesised key events in the process of ischemic retinal neurodegeneration. An interruption in the supply of blood to the retina leads to tissue ischemia which causes rapid failure of energy production and subsequent events as outlined in the figure. Key steps include the failure of the Na^+/K^+–ATPase pump, membrane depolarisation, cytoplasmic accumulation of sodium and calcium ions and the formation of destructive free radical species. The summed cellular response of these processes, if left unchecked, is cell death. This can occur by the classical and rapid necrotic process or by longer-duration apoptosis (Osborne et al., 2004).

Retina Damage in Ischemia

Glutamate is the major excitatory retinal neurotransmitter and is released in vivo, by photoreceptors, bipolar cells, and ganglion cells. The photoreceptors have only shown susceptibility to be damaged after the rest of the retina has shut down and studies have shown that ischemia has less effect on the outer layer of the retina than the inner layer of the retina (Peachey et al, 1993). The reason that photoreceptors are less sensitive remains unknown. One possibility is that their mitochondria have a high-density inner segment and is maintained on a smaller pO_2. In addition there is more neuroglobin in photoreceptors (Schmidt et al, 2003) which helps maintain oxygen levels inside cells in the cerebral and peripheral nervous system. Neuroglobin has a high affinity for oxygen and provides extra oxygen for cells under ischemic attack or in hypoxic conditions.

Ganglion cells are highly susceptible to ischemic attack and all known retinal ischemic paradigms have been described as a loss of ganglion cells (Akiyama et al, 2002; Goto et al., 2002). Ganglion cells express both the NMDA receptor and kainite type receptor (Fletcher et al., 2000), which are activated by the excessive glutamate levels, which lead to chronic depolarization. Amacrine cells also contain these receptors and are susceptible to ischemic attack through the same mechanism. Both amacrine and

ganglion cells have many metabotropic glutamate receptors which also may be the reason for their susceptibility to ischemic attack (Osborne et al., 1991).

What sets ischemic attack on the retina different from the brain, is the fact that the retina has a much longer tolerance time. A few minutes of cerebral ischemia in the human results in widespread injury, but it has been demonstrated that a primate retina can suffer up to 100 minutes of CRAO without permanent injury (Hayreh and Weingeist, 1980). This can be explained by the fact that retina contains about 100 times more neuroglobin and other energy reserves that can be used up during the hypoxic conditions of ischemia (Schmidt et al., 2003).

Alternatively there is a "no flow" phenomenon that may explain this difference (Fischer et al., 1977). This phenomenon is involved in where the rigid cranium compresses the microvasculature in a swollen brain, which causes an ongoing ischemic attack even though macroscopic blood flow is restored. In retina ischemia oedematous retina does not compress the microvasculature since there is a vitreous cavity to expand into. This large amount of time before irreversible retina destruction has allowed for many studies to be done on how to reverse ischemia or minimize ischemic damage.

Treatment Using NMDA Antagonists

It has been shown in vivo that with the addition of NMDA antagonist one can protect a neuron from ischemic attack (Lombardi et al., 1994). Competitive antagonists although shown to be effective, will only likely be used in experimental studies. These receptor antagonists may not be useful in man due to long lasting actions, which can affect neural processes. However, major interest has arisen in the use of non-competitive NMDA antagonists. This is because these compounds have shown the ability to cross blood retinal barrier and it is believed that such drugs will block the toxic actions NMDA receptors in ischemia and yet sustain NMDA receptor functions in the brain to maintain cognitive and memory processes.

This evidence supports the idea that using a NMDA antagonist one can slow down or even stop ischemic attack. One particular antagonist that has been showing great results is dextromethorphan. Dextromethorphan is an antiussive drug and is used in over the counter cough and cold medicines and it has been shown to have uses in pain relief (Bem and Peck, 1992).

Evidence on retina health after ischemic attacks were obtained through Electroretinography (ERG), which is a test in which electrodes are attached to the eye and it measures the activity of the retina by measuring the electrical activity of the retina in response to light. The ERG will contain an a-wave (initial negative deflection) followed by a b-wave (positive deflection).

In studies done using dextromethorphan while invoking a retinal ischemic attack, results show that retinas that were treated with dextromethorphan were able to maintain function better after the restoration of blood flow than retina that were not treated with dextromethorphan. In 30 and 60 minute strokes, rats that were treated with dextromethorphan had a better recovery time and less retinal damage than the mice not treated with the drug (Cao et al., 1994). However, caution is necessary when studying this drug because it may be that dextromethorphan increases cerebral blood flow and/or decrease cerebral metabolic requirements instead of blocking the ischemic stroke pathology (Osborne et al., 2004). Also it could be that dextromethorphan was only protecting the rat retinas from the increased pressure brought on by the induced stroke in the rat and therefore may not be potent for clinical use (George et al., 1988).

Dizocilpine (MK-801), another NMDA antagonist has shown better results in stopping retinal ischemia (Lam et al., 1997). However, it's neurotoxic side affects causes reduced brain function and make it almost impossible for clinical use. Also, the lowing of ischemic affects may be due to other metabolic side affects of MK-801 in that it lowers CNS temperature, which lowers the oxygen consumption during ischemia (Chi et al., 1991).

Other NMDA antagonists have been used in studies for retinal diseases. They include eliprodil, which blocks at the polyamine site of the NMDA receptor (Biton et al., 1994), fliurpitine, and memantine. Even though it has been well noted that NMDA receptor antagonists block excitoxicity, the therapeutic use should be questioned because of the negative affects that they can have on the patient. It has been suggested that memantine may be better tolerated than other NMDA antagonists because of it low affinity for the NMDA channel (Parsons et al., 1993).

Conclusion

In conclusion, ischemia in the retina, if left untreated, is almost certain to cause permanent damage to the retina and eventually to severe vision loss. Ischemia unleashes a cascade starting with the lack of oxygen, which causes the Na^+/K^+ ATPase to lose function, which leads to hyper-activation of glutamate receptors. The lack of oxygen also leads to an increase in extracellular glutamate, which can cause two forms of death. Glutamate can lead to an influx of ions, which will be followed by water under osmosis and cause cell lysis. Also, glutamate allows an increase in the uptake of calcium ions, which activates mechanisms to form radicals that can activate apoptotic mechanisms in the cell.

One proposed way of treating retinal ischemia is to administer non-competitive NMDA antagonists, which should block the entrance of calcium into the cell. The interest in using these chemicals has been big because they can cross the blood retinal barrier and are believed to not severely impair cognitive processes. Also, they can be administered after the ischemic attack has already begun. However, clinically there have been no real breakthroughs because of concerns that in vivo experiments only exhibit positive results because of other mechanisms involved and the concern of damaging normal receptor function.

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