RESEARCH ARTICLE

Opium and Opioid Receptors: From the Ancient Times to a Possible Novel Therapeutic Target for Diabetic Retinopathy

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ABSTRACT

Opium prescriptions date from the Sumerian era about 8,000 years ago, and they were commonly abused among wounded soldiers during the American Civil and Prussian French wars. With the isolation of morphine in 1805 by Setürner, the synthesis of morphine by Tschudi in 1952 and the manufacturing of synthetic derivatives called opioids, a new era of research began. In normal conditions, the endogenous opioid levels are elevated under stress conditions as a part of adaptive response. This mechanism implies in β-endorphin release, not only from the hypothalamus but also by immune circulating cells as lymphocytes. This system is powerful against pain, ischemic insult and oxidative imbalance protecting the tissues. The recognition of opioid receptors, particularly the delta subtype in retinal tissue, has broadened the potential for clinical applications. In the eye, opioid receptors were demonstrated to be present in optic nerve head, ganglion cells and pigmented epithelium cells. As such, studies have revealed that opioid receptors play a role in the pathogenesis of DR preserving the outer blood retinal barrier and also acting as a retinal neuroprotective agent. In this scenario, the modulation of the opioid receptor in the retina might become an attractive therapeutic target in the treatment of this devastating complication. Thus, this review assesses recent and scarce findings on this topic which deserves to be further investigated.

History of opioid receptors

The precise beginnings of opium's use as a drug and its connections to religion, mysticism or even recreation are uncertain, but the registration date has been identified as from ancient times, nearly 5,000 years ago. It is believed that Arab traders brought opium to India and China, and in the 10th century, opium found its way from Asia to all parts of Europe. In 1806, Setürner achieved isolation of its active compound, naming it morphine in reference to Morpheus, the God of dreams [65]. After the achievement of morphine synthesis more recently, as well as the identification of endogenous peptides with opioid receptor activity, more than 20 synthetic peptides with similar activities have been developed, all generated from three precursors (proenkephalin. prodynorphin and proopiomelanocortin). Each of these synthetic peptides acts through the transmembrane G protein receptors (opioid receptors), distributed throughout the body with different affinities, and opioid receptors that have already been cloned include the delta (δ), mu (μ) and kappa (κ) receptors and their subtypes (δ_{1-2}, μ_{1-3} and κ_{1-3}).

Physiologically, studies have shown that endogenous opioid levels are augmented under stressful conditions[2,23] through the adaptive responses to stress involving the release of β-endorphin, a small opioid peptide that is proteolytically cleaved[22] and that is primarily synthesised in the hypothalamus and pituitary[4] as well as in immune-circulating cells [6,39,43]. Macrophages, lymphocytes and monocytes represent all the components necessary for the synthesis, processing and release of β-endorphin [53] which interacts better with the μ and δ opioid receptors[59].

The increased levels of endogenous opioid peptides counteract with damaging inflammatory pathways, such as tumour necrosis factor alpha (TNF-α)[29] and nuclear kappa-light-chain-enhancer of activated B cells (NF-kβ)[1]. Unlike the other receptors, delta opioid receptors (DORs) possess unique beneficial antidepressant[14], antioxidant[58] and neuroprotective properties in the presence of cytotoxicity and hypoxia [8-9,34]. Interestingly, endogenous opioid peptide levels are reduced in patients suffering from depression or other psychological conditions [17,64], common among patients with diabetes, especially those to whom are visually under threatening [3,25,31].

Clinically, opioids are powerful analgesics, but they also produce a variety of non-analgesic effects, such as the modulation of stress responses following ischemia in brain[9,50,80,81], heart[37,45] or eye[26,51,56,62,75]. In addition, endogenous opioids (endorphins, enkephalins and dynorphins) act via specific opioid receptors distributed throughout the body, controlling the neuroendocrine axis, immunomodulation and behaviour.

DORs and the eyes

In the eyes, previous data have demonstrated the function of endogenous opioids and their receptors in the regulation of iris function, accommodative power, aqueous humour dynamics, corneal wound healing, retinal development and retinal neuroprotection [27,30,54,63,79]. Therefore, endogenous opioids and their specific receptors are involved in a wide variety of physiological and pathological processes, including dry eye, retinal ischemic diseases, glaucoma and visual accommodation. However, the mechanisms of action by which opioid receptors elicit pharmacological actions require more clarification.

DORs in an experimental glaucoma model

Glaucoma, a neurodegenerative ocular disease that irreversibly compromises vision. It is characterised by the 'cupping' of the optic nerve head (ONH) due to the loss of ganglion cells and axons, thus worsening the synapses in the lateral geniculate body. As a result, significant visual field loss is observed.

Among the described mechanisms involved in this disease are inflammation and apoptosis[20,60,71,82] of the ganglion cells, but currently, the only therapeutic strategy is to reduce intraocular pressure to slow disease progression. In the presence of glaucomatous injury, the astrocytes present in the ONH become activated, producing proinflammatory cytokines, chemokines, immune mediators, nitric oxide (NO) and reactive oxygen species, all of which act synergically towards ganglion cell death.

Husain and colleagues[29] demonstrated the presence of opioid receptors in the retina, ONH and astrocytes, and they demonstrated the effect of the systemic administration of morphine in experimental models of ischemia/reperfusion and ocular hypertension-related injury on the mitigation of retinal damage. Using the technique of electrophysiology (pattern electroretinography, which detects ganglion cell activity and enables estimation of the number of active ganglion cells), ocular hypertensive rats displayed a significant reduction in pattern electroretinogram (ERG) potentials in comparison to normal rats [1,29], indicating a significant loss of ganglion cells in the retinas of ocular hypertensive rats. More

specifically, the ocular hypertensive rats treated with SNC-121, a selective DOR agonist, promoted a sustained retinal neuroprotective effect in the animal model[1] thus preserving ganglion cell function. In conclusion, the agonism of DORs in ocular hypertensive rats is efficient in protecting the ganglion cells against hypertensive ocular conditions.

It is already known that TNF-α, an inflammatory cytokine, is associated with several neurodegenerative retinal diseases, including glaucoma, ischemic retinal diseases and diabetic retinopathy (DR)[13,16,19, 71,73,74,83], the major causes of irreversible blindness around the world[70]. For this reason, it is important to better better understand the molecular mechanisms involved, especially retinal ganglion cell toxicity and death via the TNF-a axis. Published data have elucidated the presence of TNF-α receptors in ganglion cells[42], activating inflammatory signalling and upregulating NFK-b nuclear translocation, as well as several stress-induced apoptotic transcription factors[47,33,68].

Experimental studies have demonstrated that the specific ligand for DORs, SNC-121, is efficient in preventing the upregulation and phosphorylation of STAT 3, as well as its downstream inflammatory signalling (interleukin [IL]-1β, IL-6 and TNF-α), in a model of ocular hypertensive glaucoma, thus protecting the ganglion cells from apoptosis[29]. In the same context, Husain and colleagues showed that the use of the SNC-121 DOR activator, ONH astrocytes do not produce proinflammatory IL-1β and IL-6[1]. Thus, these sets of experiments suggest the activation of DORs as a possible new neuroprotective strategy in glaucoma treatment.

Blood retinal barriers
The immune privilege of the eyes is maintained by the blood retinal barriers (BRBs), namely the inner and outer retinal barriers, both of which are functional and structural, maintaining retinal integrity. The inner BRB is comprised by endothelial cells, whose tight junctions are layered on the basal membrane and wrapped by the pericytes [5,52], multi-functional cells with plastic and regenerative potential that are pivotal in the maintenance of the neuro-glial-vascular functional retinal unit and that can dedifferentiate into myoblast or mesenchymal stem cells, [11, 72], thus enabling pathological angiogenesis[15].

The outer BRB is formed by monolayer retinal pigmented epithelium (RPE) cells with their intercellular tight junctions layered on Bruch's membrane. Recently, the outer BRB has deserved more focus in the DR field, as several studies have demonstrated the role of RPE cells in diabetic milieu conditions [67,77]. The RPE cells are highly specialised polarised cells, directing the apical polo towards the subretinal space and the basal side towards Bruch's membrane and the choroid.

Among RPE cell functions are light absorption, thus protecting the neuroretina from photo-oxidation [68] and the production of growth factors, including pigment epithelium-derived factor (PEDF)[57], vascular endothelium growth factor (VEGF) [18], transforming growth factor beta (TGF-β)[36], insulin-like growth factor-1 (IGF-1)[7] and brain-derived neurotrophic factor (BDNF), as well as proinflammatory cytokines, such as inducible nitric oxide synthase (iNOS) [21,24,38,76]. Characteristically, RPE cells play a central role in photoreceptor vitality, for which purpose they experience high autophagic activity as a mechanism of the recycling of external photoreceptor segments and the isomerization of the 11-*cis*- retinol to *trans*-retinol exchange for the proper visual cycle functioning in the photoreceptors[46,49].

The outer BRB transports water and electrolytes from the neuroretina to the choriocapillaris and glucose, ascorbic acid and fatty acids towards the neuroretina. Glucose transport is dependent on the GLUT1 and GLUT 3 receptors, which are highly diminished in diabetic conditions[32]. This is significantly deleterious to neurons, including photoreceptors, cells with the highest energy demand throughout the entire body. The water produced during photoreceptor metabolism is actively transported by the sodium–potassium pump (Na^+-K^+-ATPase), located at the apical side of the RPE cells [44], which produces an adhesive force between RPE cells and photoreceptors that is weakened or lost in diabetic conditions[11]. This later feature of the outer BRB is central to the pathogenesis of diabetic macular oedema, a primary cause of significant visual reduction among DR patients.

DOR blockage/activation in RPE cells under diabetic milieu conditions
RPE cells express iNOS, thus producing NO in response to inflammatory insults[20,38]. Hussain and collaborators[28] (2011) described that the activation of DORs inhibits the upregulation of TNF-α in the ONH, astrocytes and microglia from retinas using experimental model of ischemia/reperfusion.
Our group demonstrated the mechanism involved in outer BRB breakdown in the presence of high glucose conditions [61]. In this study, caveolin-1 (CAV-

1), a structural component of the caveolae having a lipophilic hairpin shape and embedded in the cell membrane, is implicated in the CAV trafficking of endosomes[48], and drugs[35], as well as in the regulation of tight junctions[78]. In that study, Rosales and colleagues exposed ARPE-19 cells and primary porcine RPE cells to high-glucose conditions, and after 24 h of exposure, immunofluorescence assays were performed. As expected, a monolayer of epithelial cells, tightened by the intercellular junctions and adhesion proteins, was organised, and the presence of high glucose, mimicking diabetic conditions, invoked a dramatic reduction in the Claudin-1 and Occludin expressions, two important tight junction proteins, accompanied by augmentations of the iNOS expression and NO levels. To understand further the mechanism behind tight junction reduction, we evaluated nitrosative stress and the possible S-nitrosylation of Cav-1, an important anchoring protein of the caveolae membrane structure. Under diabetic conditions, there is internalisation of Claudin-1 and Occludin through post-translational modifications to S-Cav-1.

As DORs agonists have been described to inhibit NO production via iNOS in astrocytes and microglial cells[28] under ischemia, we investigated whether DORs could act in the pathogenesis of the outer BRB in diabetic milieu conditions. ARPE-19 cells co-treated with the opioid receptor activator epicatechin, a well-known antioxidant polyphenol present in cocoa and green tea[13,41,66] and a specific DOR ligand[55]. The presence of epicatechin in retinal cells exposed to high glucose conditions prevented the production of NO-dependent iNOS, thus avoiding the S-nitrosylation of CAV-1. As a result, intercellular ARPE-19 tight junctions were maintained, either structurally and functionally, as evaluated using permeability and transcellular electrical resistance assays[61]. These compelling data identify DORs as potential therapeutic targets in the treatment/maintenance of outer BRB integrity in diabetic conditions.

DOR activation protects the retina against the toxicity from diabetes

In studying experimental models of DR, our group demonstrated evidence that the activation of DORs is beneficial in preventing the early markers of DR, such as glial fibrillary acidic protein (GFAP) and VEGF, as well as in the maintenance of the outer BRB [40]. For this study, we induced diabetes in C57BL/6JUnib mice via an intraperitoneal injection of streptozotocin. After the confirmation of the successful of the DM induction, the mice were randomized to receive not oral administration of epicatechin in drinking water. Treatment with the DOR activator epicatechin was efficient in

augmenting the DOR expression and mitigating the DR markers, namely increment of VEGF and GFAP and diminishing PEDF expressions. In order to verify whether the oral administration of epicatechin had its beneficial effect via DOR agonism, the animals were submitted to an intravitreal injection of a short hairpin RNA (shRNA) construction for the mouse retinal DOR gene. The outer BRB structure was also targeted by the DOR activator, preventing augmentation of the tight junctions Claudin-1, Occludin and ZO-1 to normal levels. The diabetic animals submitted to the intraocular transfection of DOR-shRNA did not exhibit the beneficial effects of the epicatechin as a DOR activator, meaning DOR activation might be considered a potential new therapeutic target to the treatment of DR.

Because DOR presents types 1 and 2 subtypes, we further investigated which DOR subtype activation could be beneficial in the maintenance of outer BRB properties. ARPE-19 cells were cultured and exposed to diabetic milieu and co-treated with naltrindole, a non-specific DOR blocker, in the presence of the DOR-1, D-Ala(2) and D-Leu(5)]enkephalin or DOR-2, D-Ala(2) and Deltorphin II activators. Only the DOR-1 activator was efficient in preventing the upregulation of the inflammatory signalling and functional properties of the ARPE-19 barrier under diabetic milieu co-treated with naltrindole in high glucose conditions.

To translate these experimental observations, DOR was immunolabeled in human retina. For the first time, DOR was shown to be present in RPEs and in the neuroretina of human retinal specimens, which is relevant evidence projecting DOR as a potential novel therapeutic strategy to treat the retinas of DR patients.

Concluding remarks and future directions

Nowadays, there is increasing interest in new therapeutic targets for the prevention and treatment of visual dysfunction among patients with diabetes. Although there are therapies available for diabetic eye care such as retinal laser photocoagulation, vitrectomy or even intraocular injection of anti-angiogenic drugs, diabetic vision-threatening is still a clinical challenge.

This review underlined the role and the possible beneficial effects of the delta opioid receptor in retinal tissue from experimental studies and evidences suggest that it may be relevant in human retinal tissue. The data available is scarce, thus further experimental and in vitro studies are needed in order to better understand how to control its activity in the presence of the diabetic milieu through specific ligands, avoiding extra-ocular undesirable effects.

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