Neuroprotection of the Rat's Retinal Ganglion Cells against Glutamate-induced Toxicity

Thesis

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By

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Contents

List of Abbreviations

AMPA	**:**	α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid**.**
ATP	**:**	Adenosine triphosphate.
BRB	**:**	Blood retinal barrier.
CNS	**:**	Central nervous system.
CSD	**:**	Cysteine sulfinic acid decarboxylase.
eNOS	**:**	Endothelial nitric oxide synthase.
GABA	**:**	γ-amino-butyric acid.
GCL	**:**	Ganglion cell layer.
GES	**:**	Guanidinoethyl sulfonate.
GFAP	**:**	Glial fibrillary acidic protein.
GLAST	**:**	Glutamate-aspartate transporter.
GLTs	**:**	Glutamate transporters.
GS	**:**	Glutamine synthetase.
HIOP	**:**	High intraocular pressure.
HUVECs	**:**	Human umbilical vein endothelial cells.
ILM	**:**	Inner limiting membrane.
INL	**:**	Inner nuclear layer.
IOP	**:**	Intraocular pressure.
IPL	**:**	Inner plexiform layer.
LGN	**:**	Lateral geniculate nucleus.
MSG	**:**	Monosodium glutamate.
NFL	**:**	Nerve fiber layer.
NMDA	**:**	N-methyl-D-aspartate.
NO	**:**	Nitric oxide.
OLM	**:**	Outer limiting membrane.
ON	**:**	Optic nerve.
ONH	**:**	Optic nerve head.
ONL	**:**	Outer nuclear layer.
OPL	**:**	Outer plexiform layer.
PKC	**:**	Protein kinase C.
RGCs	**:**	Retinal ganglion cells.
ROS	**:**	Reactive oxygen species.

RPE	**:**	Retinal pigment epithelium.
SYN	**:**	Synaptophysin.
Tau T	**:**	Taurine transporter.
VEGF	**:**	Vascular endothelial growth factor.

Introduction

The retina is the sensory neural layer of the eyeball. The ganglion cells of the retina are the final common pathway neurons of the retina. Their axons form the layer of nerve fibres on the inner surface of the retina. They turn tangentially to the optic disc, through which they leave the eye as fibres of the optic nerve. Thus, retinal ganglion cells (RGCs) are solely responsible for the relay of visual signals from the eye to the brain, and therefore, are of critical importance to the visual system **(Standring, 2008).**

RGCs degeneration occurs in numerous retinal diseases leading to blindness. However, no commercial drug is yet directly targeting RGCs for their neuroprotection **(Froger et al., 2012).**

Glutamate is the major excitatory neurotransmitter conducting visual signals within the retina. It is highly concentrated in the photoreceptors, bipolar cells and ganglion cells, and is released from presynaptic neurons and transmits signals upon activation of receptors [including N-methyl-D-aspartate (NMDA) receptors] in the postsynaptic neurons. However, if excessive amounts of glutamate are released or if glutamate clearance is insufficient, neuronal death can result in a process known as "excitotoxicity" **(Ishikawa, 2013).**

Glutamate-excitotoxicity has been involved in several ocular pathologies, it has also been proposed to be an important contributor to the death of RGCs in glaucoma, diabetic retinopathy, and ischemia induced by retinal or choroidal vessel occlusion **(Hernández and Simó, 2012).**

In the eye, several pathlogical conditions such as ischemia, glaucoma, and diabetic retinopathy can be mimicked by experimentally elevating extracellular glutamate concentrations or applying its analogues or sodium salt; monosodium glutamate (MSG). Moreover, they have been widely used not only to mimic the toxic effects of endogenous glutamate but to probe neurodegenerative mechanisms **(De´nes et al., 2011).**

Glutamate-induced neuronal death is initiated by increase of intracellular free calcium and sodium level followed by activation of catabolic enzymes such as proteases, phospholipases and endonucleases, protein–kinase and lipid–kinase cascades, energy compromise, and formation of reactive oxygen species (ROS) leading to cell death **(Leon et al., 2009).**

Protective agents of RGCs have been identified that include calcium channel blockers, and NMDA receptor antagonists. However, these agents are also associated with negative side effects and compliance problems. Therefore, the potential neuroprotective functions of endogenous molecules, such as taurine (2-aminoethanesulfonic acid) are being considered **(Chen et al., 2009).**

Taurine is the most abundant free amino acid in the retina and the second most abundant free amino acid in the brain after glutamate. It serves in maintaining the structural integrity of the cell membranes of the RGCs, regulating calcium binding and transport, and is considered as a neurotransmitter **(Wu and Prentice, 2010).** It has been reported to have neuroprotective effects, and has been successfully applied for the treatment of neurodegenerative diseases **(Buddhala et al., 2012).**

Reviewing the literature, no previous study could be found dealing with the neuroprotective role of taurine on the histological structure of the retina after induction of glutamate toxicity.

Aim of the Work

The aim of the present work was to show the possible neuroprotective role of taurine on the rat's retinal ganglion cells (RGCs) against glutamate-induced toxicity.

Structure of the retina

The retina is the neurosensory component of the eye; it is a thin sheet of cells, ranging from less than 100 µm at its edge, to a maximum around 300 µm at the foveal rim. It lines the inner posterior surface of the eyeball, sandwiched between the choroid externally and the vitreous body internally, and terminates anteriorly at the ora serrata **(Standring, 2008).** This portion of the nervous system processes the light information and transmits it to the brain via the optic nerve which exits the eyeball from its posterior pole. The retina detects light and sends it to the brain, but not as a simple point-by-point representation of the image. In the retina, complex processes are carried out before sending the information to superior centers **(Mendez-Vilas and Diaz, 2010).**

When viewed with an ophthalmoscope to show the fundus oculi, the most prominent feature is the blood vessels emanating from and entering the optic disc. Centred temporal and inferior to the optic disc lies the macula (approximate diameter 5–6 mm), the middle of which is composed of the fovea and foveola, and easily identified with an ophthalmoscope as an avascular area with a yellow tinge **(Fig.I) (Standring, 2008).**

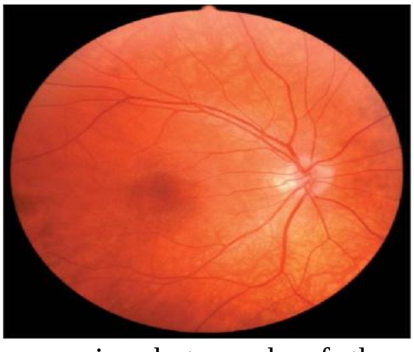

Fig. (I): Ophthalmoscopic photograph of the right human retina **(Standring, 2008).**

Both human and rat retina consist of the same histological layers **(Suckow et al., 2005).** Similarities in the physiology and cell biology of retina in humans and rats make rats a valuable model to study **(Bonilha, 2008).**

The retina is composed of a variety of epithelial, neural and glial cell types whose distribution conventionally divides it into 10 layers **(Fig.II) (Standring, 2008).**

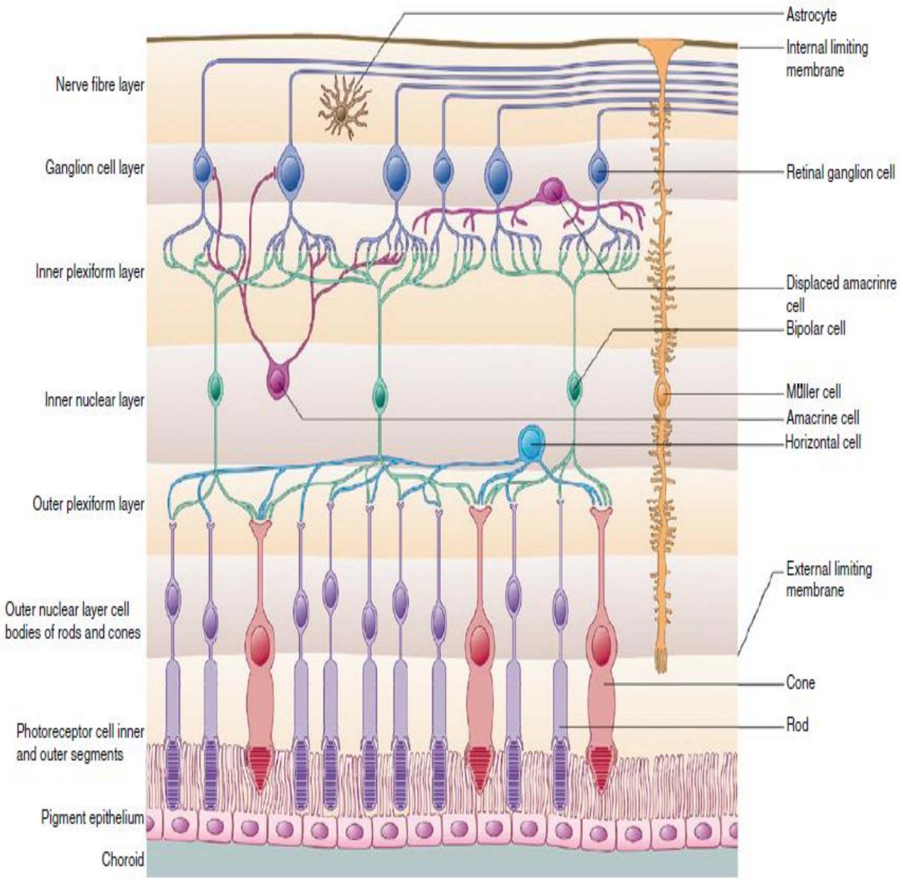

Fig. (II): Diagram showing neuronal cell bodies and their processes in the different layers of the retina **(Standring, 2008).**

The cellular elements of the retina are arranged and adapted to meet the functional requirements of the different regions of the retina. They are organized into **four main layers;** i. the photoreceptor layer, containing photoreceptor outer and inner segments, ii. the outer nuclear layer, containing the photoreceptor nuclei, iii. the inner nuclear layer, containing diverse kinds of information processing neurons and iv. the ganglion cell layer (GCL), containing ganglion cells. These neurons make synaptic connections in two separate layers, the outer plexiform layer (OPL), where photoreceptor, horizontal and bipolar cells are interconnected, and the inner plexiform layer (IPL), where the processes of amacrine and bipolar cells make synapses with ganglion cell dendrites **(Nag and Wadhwa, 2012).**

Visual perception is a sensory process initiated at the retina, and completed in the cerebral cortex. Two main functions are currently performed by the retina: 1) the initial conversion of light energy into electric signals, "*phototransduction*", which is carried out by photoreceptors; 2) a series of physiological processes performed by retinal interneurons (bipolar, horizontal and amacrine cells), in order to encode the different attributes of the visual stimuli (shape, movement and color) in electrical signals **(Mendez-Vilas and Diaz, 2010).**

The basic system of retinal information processing consists of a direct pathway of visual information that flows from photoreceptors to bipolar cells to ganglion cells. The ganglion cells fire action potentials in response to light, and these impulses propagate down the optic nerve to the projection nuclei and visual areas in the brain where visual processing is completed. This direct pathway is influenced by two transverse fluxes of modulatory signals coming from horizontal cells in the OPL and amacrine cells in the IPL. Horizontal cells receive input from the photoreceptors and project their processes laterally to influence surrounding bipolar cells. Amacrine cells receive input from bipolar cells and project their processes laterally to influence surrounding bipolar and ganglion cells. Both, horizontal and amacrine cells usually make electrical and chemical synapses with neighbor cells of the same type. Therefore, from the photoreceptors, neural activity flows radially to bipolar and ganglion cells, and laterally via horizontal cells in the outer retina and amacrine cells in the inner retina **(Mendez-Vilas and Diaz, 2010).**

Although most neural activity flows from the photoreceptors towards the brain, some information flow occurs in the opposite direction via centripetal fibres in the optic nerve and interplexiform cells in the retina which connect the inner and outer plexiform layers **(Standring, 2008).**

The classic ten layered appearance of the retina is absent in the optic nerve head, the fovea and foveola, and the ora serrata. At the optic nerve head, the axons of the retinal ganglion cells leave the retina to form the optic nerve and all the other neural cell types of the retina are missing. At the fovea and foveola, the inner five layers of the retina are ‘pushed aside' **(Standring, 2008).** The ora serrata delineates the anterior termination of the sensory retina and the beginning of the pars plana of the ciliary body. At this junction, the sensory retina is reduced to a single cell layer which, anteriorly, becomes the nonpigmented ciliary epithelium whereas the retinal pigment epithelium (RPE) is replaced by pigmented ciliary epithelium **(Reynolds and Olitsky, 2011).**

Histological layers of the retina:

Layer 1: Retinal pigment epithelium (RPE):

The RPE, is composed of cuboidal cells that form a single continuous layer extending from the periphery of the optic disc to the ora serrata, where it continues as the outer ciliary epithelium. Their cytoplasm contains numerous melanosomes. The density of RPE cells is greater in the fovea than in the periphery **(Kaufman and Alm, 2003).**

In the central retina, where RPE cells are most tightly packed, they form a single layer of cuboidal epithelium. Tight junctions (Zonulae occludenes) between adjacent RPE cells form the outer blood-retina barrier, an important physiologic barrier to the free flow of molecules between the leaky choriocapillaris and the photoreceptors of the neuroretina **(Cunha-Vaz, 2004).**

Cellular polarity and the abundance of mitochondria, endoplasmatic reticulum, and free ribosomes all indicate a very high level of metabolic activity in the RPE cell. Melanin renders the RPE dark brown to black. Light reaching the outer retina but missing the photoreceptors is absorbed by the RPE, which, like melanin elsewhere in the eye, prevents such stray light degrading image quality. The RPE is required for the regeneration of bleached visual pigment and may have antioxidant properties. It also secretes a variety of growth factors necessary for the integrity of the choriocapillaris endothelium and the photoreceptors, and produces a number of immunosuppressive factors. A failure of any of the diverse functions of the RPE could result in compromised retinal function and eventual blindness **(Strauss, 2005).**

Infoldings of the basal and apical surfaces greatly increase the RPE surface area, facilitating active transport across its cell surface with both the choriocapillaris and the photoreceptor layer **(Ryan, 2013).** Apically towards the photoreceptors (towards the rods and cones), the cells bear long microvilli which contact, or project between, the outer segments of rods and cones. The tips of rod outer segments are deeply inserted into invaginations in the apical membrane of the RPE **(Fig. III).** RPE cells play a major role in the turnover of rod and cone photoreceptive components. Their cytoplasm contains the phagocytosed tips of rods and cones undergoing lysosomal destruction. The final products of this process are lipofuscin granules, which accumulate in these cells with age **(Standring, 2008).**

During embryogenesis, the central neuroectoderm of the optic vesicle invaginates to form the inner leaf of the optic cup and becomes the neurosensory retina. The peripheral neuroectoderm of the optic vesicle forms the pigmented outer leaf of the optic cup and differentiates into the retinal pigment epithelium (RPE). As a result of this invagination; the neuroretina and the RPE become apposed, and the attachments between them become unsupported by junctional complexes, being only separated by a potential subretinal space, which can be filled with fluid, therefore, they can be easily separated in the clinical condition of retinal detachment arising from trauma or disease **(Reynolds and Olitsky, 2011).**

The RPE is separated from the choriocapillaries by **Bruch's membrane**. It is an elastic membrane composed of five layers: the basement membrane of the choriocapillaris, an outer collagenous layer, a central elastic layer, an inner collagenous layer, and the basement membrane of the RPE. It stretches from the optic disc at the posterior pole to the ora