CHARACTERIZATION OF Taurine UPTAKE IN THE RAT RETINA

1Julius D. Militante and 1,2John B. Lombardini
Departments of Pharmacology1 and Ophthalmology & Visual Sciences2, Texas Tech University Health Sciences Center, Lubbock, Texas, 79430, USA

INTRODUCTION

Taurine is a free amino acid found in mammalian tissues and its pharmacological depletion in various animal models has been demonstrated to cause visual deficits and morphological degeneration in the retina1. Taurine is an important modulator of cellular processes whose most unique characteristic is the unusually high mM concentrations in which it is found intracellularly2. In the retina, concentrations as high as 79 mM have been measured3 with most of the taurine being exogenous in nature1. The steep gradient across the cell membrane requires a very efficient transport system that is carefully regulated.

Taurine uptake in the retina has been demonstrated, in various species, to have 2 saturable components of differing affinity to taurine2. The transport systems exhibit a 10- to 100-fold difference in their affinities for taurine, as measured by the taurine concentration at which a half-maximal velocity is attained \( K_M \). In the retina, visual transduction is initiated in the rod outer segments (ROS) of the photoreceptor cells4 to which the high-affinity taurine uptake component has been specifically linked5,6. However, the kinetics of taurine uptake in the ROS have not been studied in detail. In view of the
visual deficits associated with pharmacological inhibition of taurine uptake, particularly with the taurine analogue guanidine-ethanesulfonate (GES), the study of uptake kinetics in the ROS is crucial in the understanding of the role of taurine in the visual transduction process.

In rat astrocytes and human GL15 glioma cells, activation of protein kinase C (PKC) by phorbol myristate acetate (PMA) results in the inhibition of taurine uptake\textsuperscript{7,9}, suggesting that PKC modulation of taurine transport may be important in the central nervous system. Chelerythrine (CHT) is a potent PKC inhibitor that has been found to antagonize the effects of taurine in the retina\textsuperscript{10,11}. This study aims to characterize taurine uptake in the retina, particularly in the ROS, in terms of transport kinetics and PKC regulation, using pharmacological agents such as GES and CHT.

**MATERIALS AND METHODS**

**Materials**

Radiolabelled \[^{3}H\]taurine was purchased from New England Nuclear. Chelerythrine chloride (CHT) was obtained from LC Laboratories. Phorbol myristate acetate (PMA) and staurosporine (STAU) were purchased from Sigma.

**Preparation of tissue samples**

Whole retinal homogenate and isolated ROS preparations were obtained according to previously established protocols\textsuperscript{12}. Briefly, retiniae were dissected from frozen adult rat eyes, washed and pooled in Krebs-bicarbonate-Ringer (KBR) solution, maintained on ice. The retiniae were homogenized with a handheld glass mortar and pestle in KBR solution.

To prepare isolated ROS, the retiniae were vortex-mixed gently for 10 seconds to detach the ROS. The tissue and cell debris were allowed to settle and the Supernatant containing the suspended ROS was collected. The procedure was repeated with the pelleted retiniae to maximize the recovery of ROS. The pooled supernatant was centrifuged and the pelleted ROS was resuspended in KBR solution.

**Taurine uptake assay**

The assay used in measuring taurine uptake was performed as previously described\textsuperscript{13}. Briefly, the reaction was performed in KBR solution and for uptake kinetic experiments, unlabelled taurine was added in varying