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Imidazole-4-acetic acid, a new lead structure for interaction with the taurine transporter in outer blood-retinal barrier cells

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ABSTRACT

Retinal diseases leading to impaired vision and ultimately blindness are mainly characterized by ischemic and hypoxic stress. Targeting the retinal ρ-containing γ-aminobutyric acid type A receptors (ρ $GABA_A$Rs) and thereby decreasing the retinal neuronal activity has been proposed as a novel therapeutic approach. The taurine transporter (TAUT) plays a key role in the retinal transport of GABA and has been previously suggested to display a higher functional activity in the retina compared to the brain. TAUT would therefore stand as a suitable target for the selective delivery of ρ $GABA_A$R ligands into the retina. Consequently, an *in vitro* model of TAUT at the outer blood-retinal barrier (BRB) was developed and characterized using the ARPE-19 cell line. Furthermore, the structural requirements of $GABA_A$R ligands for interacting with TAUT at the BRB were investigated for a series of standard $GABA_A$R ligands by testing their ability to inhibit the TAUT-mediated influx of taurine in ARPE-19 cells. Results showed that taurine influx was seven-fold higher when the ARPE-19 cells were cultured under hyperosmotic conditions and was demonstrated to display saturable kinetics ($K_m = 27.7 \pm 2.2$ μM and $J_{max} = 24.2 \pm 0.6$ pmol/cm^2·min). Furthermore, the taurine influx was significantly inhibited in a concentration-dependent manner by GABA and imidazole-4-acetic acid (IAA), which is a naturally occurring metabolite of histamine. These compounds display similar K_i values of 644.2 μM and 658.6 μM, respectively. Moreover, IAA demonstrated higher inhibitory properties than the other tested GABA analogs: 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol (THIP), 4,5,6,7-tetrahydropyrazolo[5,4-*c*]pyridin-3-ol (Aza-THIP), muscimol, and thiomuscimol. These studies demonstrated that IAA interacts with TAUT, which makes IAA a new lead structure in the development of new compounds, which are not only interacting with TAUT but also potent ρ $GABA_A$R ligands.

1. Introduction

Retinal diseases leading to impaired vision and ultimately blindness include disorders such as age-related macular degeneration (AMD), optic neuropathy, diabetic retinopathy, and vascular occlusion, that are all related to ischemic and hypoxic stress and are implicated in loss of retinal ganglion cells (Friedman et al., 2004; Ramsey et al., 2007). Among these, AMD and diabetic retinopathy are the leading causes of blindness in the developed countries. There is currently no effective cure to retinal hypoxic stress but a proposed strategy is to decrease the neuronal activity and thereby reduce the metabolic demands of the retina (Hanus et al., 2016; Qian and Ripps, 2011; Wong et al., 2012).

The majority of the inhibitory control in the retina is mediated by the ionotropic ρ-containing γ-aminobutyric acid type A receptors (ρ $GABA_A$Rs), also known as the $GABA_C$ receptors (Lukasiewicz et al., 2004). The ρ $GABA_A$Rs are mainly found in the axons of bipolar cells, which transmit signals from the photoreceptors (rods and cones) to the ganglion. The unique properties and localization of the ρ $GABA_A$Rs in the retina make this type of receptors a particular suitable target for regulating retinal neuronal activities (Bormann, 2000).

Although ρ $GABA_A$Rs are less abundant in the brain compared to retinal tissues, they have been identified in some regions of the central nervous system (CNS) where they have been proposed to be involved in processes connected to regulation of sleep and cognition (Arnaud et al., 2001; Johnston et al., 2003). Therefore, selective delivery of ρ $GABA_A$R ligands to the retina is an important issue. Compared to topical eye drops or intravitreal drug administration, oral and parenteral administration *via* the systemic blood circulation could constitute an advantage in retinal drug delivery. Indeed, topical administration by eye drops can generally not generate sufficient therapeutic concentrations in the retinal tissues due to low corneal and conjunctival absorption (<5%) as well as tear and aqueous humor drainage while intravitreal injections are invasive methods that may cause retinal detachment or endophthalmitis (Janoria et al., 2007; Urtti, 2006). However, drug delivery from the central circulating blood to the posterior segment of the eye

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remains challenging as the retina is separated from the circulating blood by the blood-retinal barrier (BRB), which consists of retinal capillary endothelial cells (inner BRB) and retinal pigment epithelial cells (outer BRB) that are connected by tight junctions. The BRB hampers non-specific transport between the circulating blood and the neural retina. Thus, membrane transporters at the BRB play a key role in the transport of nutrients to the neural retina and removal of neurotransmitters and their metabolites (Hosoya and Tomi, 2005). Drug substances designed as substrates to membrane transporters in the BRB could possibly be delivered from the circulating blood to the retinal ρ $GABA_A$Rs. Relevant retinal transporters for the influx of γ-aminobutyric acid (GABA, Fig. 1) analogs include GABA transporters (GATs) and the taurine transporter (TAUT, SLC6A6), which are mainly responsible for the transport of GABA at the retina (Honda et al., 1995; Tomi et al., 2008). TAUT is a high-affinity sodium dependent transporter playing a key role in the transport of taurine (Fig. 1) and also GABA at both the inner and the outer BRB (El-Sherbeny et al., 2004; Lambert et al., 2015; Tomi et al., 2007). It has previously been estimated that the transport of [^{3}H]taurine from the circulating blood to the rat retina is almost 30 times higher than the corresponding value across the blood-brain barrier (BBB), which could indicate a higher expression of TauT in the rat retina compared to the brain (Tomi et al., 2007).

Studies have shown TAUT to be expressed in ARPE-19 cells, a spontaneously arising human retinal pigment epithelial (RPE; outer BRB) cell line (El-Sherbeny et al., 2004). The RPE is responsible for the nourishment of one third of the retina (the inner BRB is responsible for the remaining two third of the retina) (Hosoya and Tomi, 2005). Essential nutrients for photoreceptors, where ρ $GABA_A$Rs have been shown expressed, are mostly supplied across the outer BRB (Pattnaik et al., 2000). Although the circulating blood supplies the retina across both the inner and the outer BRB we here have selected the ARPE-19 cell line for developing an *in vitro* model of TAUT at the outer blood retinal barrier.

To investigate the structural requirements of ρ $GABA_A$R ligands for interacting with TAUT at the BRB we here report the development of a robust *in vitro* model for studying TAUT in retinal cells and the inhibitory potencies of a series of standard $GABA_A$R ligands on taurine influx. These studies will provide first data for the further design of new potent ρ $GABA_A$R ligands that also interact with TAUT.

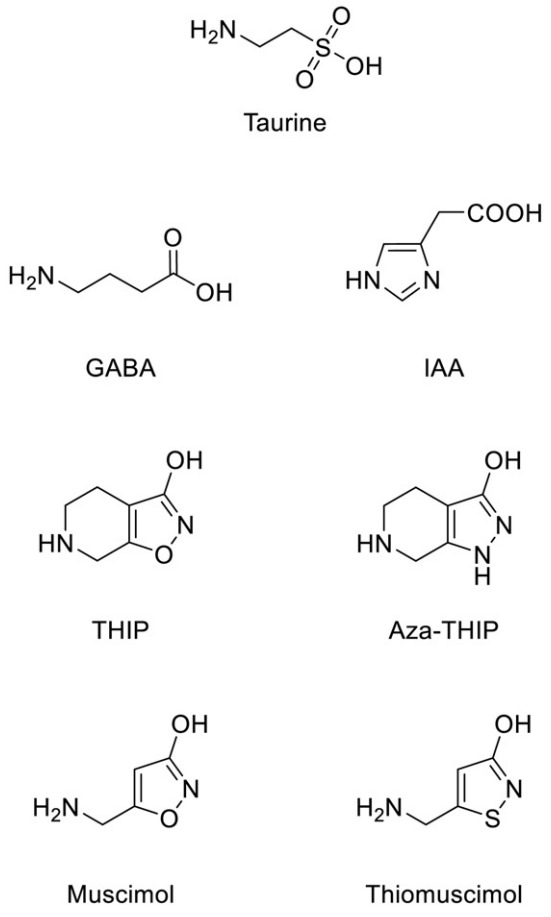

Fig. 1. Structures of taurine and the selected standard $GABA_A$R ligands for inhibition of taurine influx in the ARPE-19 cell line.

2. Experimental section

2.1. Materials

2,2-^{3}H-taurine ([^{3}H]taurine; 22.7 Ci/mmol, 1 mCi/mL) was acquired from Perkin Elmer (Boston, MA, USA). GABA and non-tritium labeled taurine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Imidazole-4-acetic acid (IAA) was a kind gift from Niels Clauson-Kaas A/S Chemical Research Laboratory (Farum, DK). 4,5,6,7-Tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol (THIP), 4,5,6,7-Tetrahydropyrazolo[5,4-*c*]pyridin-3-ol (Aza-THIP), muscimol, and thiomuscimol were provided by the Department of Drug Design and Pharmacology, University of Copenhagen, and synthesized by Frølund and co-workers as previously described (Krogsgaard-Larsen, 1977; Krogsgaard-Larsen and Christensen, 1976; Krogsgaard-Larsen and Roldskov-Christiansen, 1979; Lykkeberg and Krogsgaard-Larsen, 1976). Hank's balanced salt solution (HBSS) (10×) and sodium bicarbonate (7.5%) were purchased from Gibco, Invitrogen (Paisley, UK). 3-[[1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl]amino]-2-hydroxypropane-1-sulfonic acid (TAPSO) and 10× trypsin-EDTA were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ultima Gold scintillation liquid was provided by Perkin-Elmer (Boston, MA, USA). Water used for the experiments was provided by a Mili-Q water purification system. Chemicals needed for cell cultivation (Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F12)), 100× penicillin/streptomycin, 100× L-glutamine, 7.5% $NaHCO_3$, 10× trypsin-EDTA, 1× Dulbecco's Phosphate Buffered Saline (without Ca^{2+} and Mg^{2+}), and D-(+)-raffinose pentahydrate were purchased from Sigma-Aldrich (St. Louis, MO, USA) except from fetal bovine serum (FBS), which was purchased from Fisher Scientific (Waltham, MA, USA). Cell culture plastic wares and 24 well plates were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Cell cultivation

The spontaneously arising retinal pigment epithelial cell line derived in 1986 by Amy Aotaki-Keen (ARPE-19 cells) was from American Type Culture Collection (ATCC; Manassas, VA, USA). It was cultured in a DMEM/F12 medium with 100 units/mL and 0.1 mg/mL of penicillin/streptomycin, 2 mM L-glutamine, 0.75 mg/mL $NaHCO_3$, and 10% FBS in a humidified incubator at 37 °C with 5% CO_2. The culture medium was changed every other day and the cell line was sub-cultured once a week. The cells were used for experiments in passage width of 1–16 passages after thawing. The cells were seeded at the bottom of 24 well plates (1.9 cm^2 growth area) with a seeding density of 40.000 cells/cm^2 and were grown to confluent monolayers for 14 days before they were used for experiments. ARPE-19 cells were cultured with the above described growth media that was supplemented with D-(+)-raffinose pentahydrate, in a final concentration of 500 mOsm, 24 h prior to the influx experiments except for the one performed without raffinose.

2.3. Experimental procedure for influx studies

Influx studies were performed at pH 7.4 in Hank's Balanced Salt Solution (in mM: $CaCl_2$, 1.26; $MgCl_2$ 0.49; $MgSO_4$, 0.41; KCl, 5.33; KH_2PO_4,