Study of taurine and tauret content in the compound eye of locust with light and dark adaptation

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Summary. Taurine as well as tauret (retinyliden taurine) levels were measured in locust Locusta migratoria compound eyes. HPLC measurements revealed relatively low taurine levels (1.9 ± 0.16 mM) in dark-adapted eyes. Glutamate, aspartate and glycine levels were 2.0 ± 0.2, 2.7 ± 0.4 and 3.0 ± 0.37 mM, respectively, while GABA was present only in trace amounts. After about 4 h of light adaptation at 1500–2000 lx, amino acid levels in the compound eye were as follows: taurine, 1.8 ± 0.17 mM; glutamate, no change at 2.1 ± 0.2 mM; aspartate sharply increased to 4.7 ± 0.7 mM; glycine slightly decreased to 2.8 ± 0.3 mM; and GABA trace levels. In the compound eye of locust Locusta migratoria, the existence of endogenous tauret in micro-molar range was established. In the dark, levels were several times higher compared with compound eye after light adaptation 1500 lx for 3 h, as estimated by TLC in combination with spectral measurements. Existence of tauret in compound eye is of special interest because in the compound eye, rhodopsin regeneration is based on photoregeneration.

Keywords: Taurine – Tauret – Compound eye – Aspartate – Glutamate glycine

Introduction

After the 1970s, the range of biological phenomena associated with taurine has progressively expanded to include pregnancy, birth, development, brain, heart, liver, and kidney function, osmoregulation, antioxidant and membrane stability (see Huxtable and Sebring, 1986; Huxtable, 1990; Van Gelder, 1990). With time, it became evident and currently there is no doubt that taurine plays an essential role in vision in vertebrates (Pasantes-Morales et al., 1989; Lima et al., 1990, 2004; Petrosian and Haroutounian, 1990, 2000; Lombardini, 1991; Militante and Lombardini, 2002). First of all, a high level of taurine, up to 40 mM concentration, is characteristic for the retina of all vertebrates tested, even for those species which have low taurine content in other tissues and organs (Orr et al., 1976; Voaden et al., 1977; Lima et al., 1990, 2004; Petrosian et al., 2006). The highest level of taurine in the retina (50–80%) has been observed in the photoreceptors (Orr et al., 1976; Voaden et al., 1977; Lake and Vardone-Smith, 1989; Lima et al., 1990). The importance of taurine for vision of vertebrates was emphasized after 1975 when it was recognized that a taurine-free diet causes blindness in cats (see Hays et al., 1975; Schmidt et al., 1977). For visual systems based on wave-guide optic principles such as that specific to insect compound eyes (see Gribakin and Govardovski, 1975), rhodopsin regeneration is based on photoisomerization (Smith and Goldsmith, 1991), and for these systems, there are only rare reports about taurine content. Taurine like immunoreactivity was noted in compound eye of Drosophila and Locusta (Bicker, 1992; Pirvola and Panula, 1992) and Honeybee (Schaffer et al., 1988; Eichmuller and Schaffer, 1995). However, there are almost no direct measurements of tissue levels of taurine or its derivatives in the compound eye.

The other point of interest is tauret (retinyliden taurine), an endogenous taurine conjugate and newly discovered in vertebrate retinae and RPE cells (Petrosian and Haroutounian, 1990, 2000; Petrosian et al., 1996, 2000a, b, 2006). It was suggested that taurine associates with specific retinoid binding proteins and plays an essential role in 11-cis and all-trans retinoid transport between RPE and retina, and that this mechanism involves tauret. Thus, tauret may be involved in rhodopsin regeneration and prevention of light induced damage of photoreceptor cells (Petrosian and Haroutounian, 1990, 2000; Petrosian et al., 1996). Until now, there is no data concerning the existence of tauret in the compound eye where, unlike vertebrate retinal rods, rhodopsin regeneration
is based exclusively on photoregeneration. In the current study, along with efforts to measure taurine in compound eye under dark and light adaptation, we also tried to estimate whether there exists endogenous tauret in the compound eye of locust *Locusta migratoria*.

### Materials and methods

#### Light adaptation

In this study, we used crickets *Locusta migratoria* which were collected not far from Yerevan, in the Eghward region, during September and October of 2004 and 2005. Some groups of the insects were dark adapted for 14–20 h at 20°C for further determination of taurine, glycine, GABA, aspartic and glutamic acid levels as well as tauret levels. The other groups were light adapted at 1500–2000 lx during 3–5 h at 20°C for the same purpose.

#### Chemicals

Methanol, HPLC grade, was obtained from Roth (Germany). O-phthalaldehyde and standards of aspartate (1 mg/ml), glutamate (5 mg/ml), glycine (1 mg/ml), taurine (1 mg/ml) GABA (1 mg/ml) and homoserine (1 mg/ml) were obtained from Biokhrom (Russia). All-trans tauret was synthesized in a one step reaction for use as a standard.

#### HPLC and TLC analysis of endogenous neuroactive amino acids

Compound eyes of crickets *Locusta migratoria* were prepared for dark or light adaptation under dim red or white 15000 lx, respectively. Samples, each containing 6 compound eyes, were immediately weighed to avoid loss of mass. Then, the samples were homogenized by glass–glass homogenizer in 300 ml to avoid loss of mass. Then, the samples were homogenized by glass–glass homogenizer in 300 ml. Then, the samples were centrifuged at 15000 rpm for 15 min. After o-phthalaldehyde derivatization, 50 μl of the supernatants were used for amino acid content determination by high-pressure liquid chromatography (ED-108, Biokhrom, Russia). Homoserine was used as an internal standard for amino acid quantification. Column C18 150 × 4.6 (Phenomenex) was employed for amino acid separation. Taurine content was also estimated in parallel with TLC. TCL was performed on starch pre-coated silica gel plates (Armsorb). For this procedure, 3 μl 0.1 N HClO4 extracts and standard synthetic taurine and other amino acids solutions were spotted on Armsorb sheets which were then developed for about 2 h in butanol:acetic acid:water combined in a 36:9:15 ratio. After development, spots on sheets were treated with a acetone:acetic acid mixture in a 36:4 ratio plus 100 mg ninhydrin, then were visualized by heating at about 120°C for 5–6 min.

#### Estimation of tauret content

After collecting in conditions described above, compound eyes were rapidly frozen in dry ice. Then, 6 samples of dark and light adapted compound eyes, each sample containing 12 compound eyes, were freeze dried (freeze-dryer LGA 05, WML, Germany) at −20°C under vacuum (1 × 10−3 Pa) for about 30 h. The freeze-dried samples were weighed and homogenized, and 100 μl dry methanol was added to the homogenates. After 2 h of extraction, samples were centrifuged (15 min at 15000 rpm) and methanol excess was evaporated until about 30 μl supernatant remained which was then further analyzed. TCL of the samples was performed in combination with spectral measurements. For this purpose, 30 μl of 100% methanol extracts of dried compound eye and 30 μl of standard synthetic tauret (1 mM in 100% methanol) were spotted on the Armsorb sheets. These were then developed for about 20 min with the mixture of chlorophrom:methanol:trifluoroacetic acid in a ratio 20:6:0.4.

In some cases during development, trifluoroacetic acid was removed from the developing system. After development, Armsorb sheets were cut into separate strips. Then strips with spots corresponding to tauret standards (Rf equal to about 0.65) were placed into 1.2 ml of 100% methanol solution for compound extraction. Then absorbance spectra of methanol extracts of such spots were recorded. In case of development without trifluoroacetic acid, absorbance was recorded twice, before and after protonation, by adding 10 μl 0.1 N HCl. In this way one can establish whether there exists Schiff base bearing molecule in samples such as tauret. Absorbance was recorded on spectrophotometer Specord (Carl Zeiss Jena) or Hitachi 150-20 (wavelength range 334 to 500 nm).

### Results

#### TLC estimation of taurine in locust compound eye

The first series of TLC using taurine standards revealed relatively high levels of taurine in locust compound eyes kept in the dark, roughly estimated to be in the range 10–20 mM. It is interesting to note that there is a noticeable decrease in taurine levels after 1500–3000 lx/3 h light adaptation (see Fig. 1) in each series of TLC determination. Another TLC study of cricket compound eyes was performed using different amino acid standards. Rather high levels of glutamate, aspartate and glycine, as compared with taurine, and relatively low GABA content was revealed with dark adaptation (Fig. 2). During this series of TLC determinations, it was difficult to get any quantitative estimation of taurine content. In the same series, a decrease in taurine content in the cricket compound eye and an increase in aspartate and glutamate (Fig. 2) were noted with light adaptation at 1500–2000 lx/3 h.

![Fig. 1. TLC determination of taurine content in the locust compound eye. First 5 spots are taurine standards 0.25, 0.5, 1.0, 2.5, 5.0 mM. Samples of compound eye in dark (D) under light (L) and extract from head (H) from dark-adapted insect](image)