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Research Report

Spontaneous and visible light-induced ultraweak photon emission from rat eyes☆

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ABSTRACT

Here, we present the first experimental *in vitro* evidence of the existence of spontaneous and visible light-induced ultraweak photon emission from freshly isolated whole eye, lens, vitreous humor, and retina samples from rats. These results suggest that the photochemical source of retinal discrete noise, as well as retinal phosphenes, may originate from natural bioluminescent photons within the eyes. During normal vision, the eyes are continuously exposed to ambient powerful photons that pass through various parts of the eyes, which can produce ultraweak delayed bioluminescent photons that arise from diverse parts of the eyes. Although the importance and possible role of ambient light-induced permanent delayed photons (within different parts of the eyes) during vision requires further investigation, our study may provide evidence of an origin of discrete dark noise and retinal phosphenes.

1. Introduction

During natural metabolic processes, continuous and spontaneous ultraweak photon emissions (UPEs) have been observed without any excitation from any kind of living organisms or cells (Quickenden and Que Hee, 1974; Tilbury and Cluickenden, 1988; Scott et al., 1991; Devaraj et al., 1991; Takeda et al., 1998, 2004; Kobayashi et al., 1999a; Kobayashi and Inaba, 2000; Niggli et al., 2001; Nakano, 2005; Mansfield, 2005; Yoon et al., 2005; Van Wijk et al., 2006; Laager et al., 2008; Tafur et al., 2010). UPEs of biological systems are very weak electromagnetic waves in the optical range of the spectrum. These spontaneous photon emissions are known by many names including biophotons, low-intensity chemiluminescence, dark luminescence, ultraweak electromagnetic light, ultraweak bioluminescence, ultraweak autoluminescence, and ultraweak photons. UPEs cannot be seen by the naked eye but can be measured by very sensitive instruments, such as a photo-

☆ Delayed luminescence (DL) is the long-term ultraweak re-emission of optical photons from diverse cells, organisms, and other material if they were illuminated with monochromatic or white light (Ho et al., 2002; Popp and Yan, 2002; Kim et al., 2005). DL intensity is radically lower than the well-known fluorescence or phosphorescence. The decay time of DL is dependent on the physiological conditions of the samples and the kinds of tissues they were extracted from, as well as the intensity, duration, and spectral distribution of illumination (Kim et al., 2005).

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Abbreviations: UPE, ultraweak photon emission; PMT, photomultiplier tube; EM-CCD, electron multiplier CCD camera; ROS, reactive oxygen species; RNS, reactive nitrogen species; EEG, electroencephalogram; cGMP, cyclic guanosine monophosphate; DC, direct current; V1, primary visual cortex; DL, delayed luminescence

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multiplier tube (PMT) or an electron multiplier CCD (EM-CCD) camera, as well as by in situ biophoton autography (Sun et al., 2010).

UPEs are attributable to the endogenous production of diverse biochemical reactions, especially bioluminescent radical reactions of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and their derivates, as well as the simple cessation of excited states during natural oxidative processes. There are several possible sources of UPEs within cells including mitochondrial respiration chains, lipid peroxidation, peroxisomal reactions, oxidation of catecholamines, and oxidation of tyrosine and tryptophan residues in proteins (Kruk et al., 1989; Watts et al., 1995; Nakano, 2005; Catalá, 2006). These ultraweak photon emissions are comprised of various ranges of wavelengths including infrared, visible, and ultraviolet ranges. UPEs originate mainly from mitochondrial oxidative metabolism and lipid peroxidation that produce light-emitting molecules such as triplet carbonyls and singlet oxygen (Nakano, 2005; Thar and Kühl, 2004).

Neural cells also generate constantly ultraweak photons during their natural metabolism (Isojima et al., 1995; Kobayashi et al., 1999a, b). *In vivo* UPEs from rat brains are associated with electroencephalogram (EEG) activity, cerebral energy metabolism, and oxidative processes (Kobayashi et al., 1999b). UPEs from neural tissue depend on the membrane depolarization and Ca^{2+} entry into cells (Kataoka et al., 2001). Neural activity-dependent UPEs have been demonstrated in the rat hippocampus (Kataoka et al., 2001). Recently, additional evidence by Sun et al. (2010) revealed that ultraweak bioluminescent photons can conduct along the neural fibers and can be considered a means of neural communication. They have also been suggested that biophotonic and bioelectronic activities are not independent biological events in the nervous system, and their synergic action may play an important role in neural signal transductions.

Recently, Bókkon (2008) proposed a novel biophysical (*redox molecular*) interpretation of phosphene lights. He argued that phosphenes could arise from unregulated overproduction of free radicals and excited biomolecules in various parts of the visual system (Bókkon, 2008). Unregulated overproduction of free radicals and excited species can generate a brief increase of UPEs in different regions of the visual system, and if this excess of UPEs exceeds a threshold, they can appear as phosphene lights in our mind. Among those phosphenes that share this common feature are electrical-, magnetic-, ionizing radiation-, and mechanical-induced phosphenes, as well as phosphenes arising from exposure to various drugs, stress, and optic nerve diseases (Bókkon, 2008). In addition, Bókkon and Vimal (2009) suggested that the discrete dark noise of rods (*in dark-adapted retinal cells*) may result from the bioluminescent photons generated continuously by retinal lipid peroxidation and oxidative metabolism.

According to electrical recordings, rods have two components of the dark noise: a constant, low-amplitude component (*amplitude*≈*0.2 pA*) and a discrete component (*amplitude*≈*1 pA*) (Schwartz, 1977; Baylor et al., 1980). The continuous component of a rod's noise originates from the spontaneous activation of cGMP phosphodiesterase molecules (Rieke and Baylor, 1996). Spontaneous activation of rhodopsin produces discrete dark noises indistinguishable from single-photon responses (Baylor et al., 1980). In a retinal photoreceptor cell, a visual pigment molecule can be activated not only by absorption of a photon but also by thermal energy. Current estimates of the activation energies for these two processes in vertebrate rod and cone pigments are on the order of 40–50 kcal/mol for activation by light and 20–25 kcal/mol for activation by heat (Ala-Laurila et al., 2004).

To resolve this discrepancy, it was suggested that photon and thermal activation of rhodopsin follow different biochemical processes. The most persuasive argument for a separate low-energy thermal pathway stems from the observation that the discrete noises in rods occur in a small subpopulation of rhodopsins, where the Schiff base linking the chromophore to the protein part has been deprotonated (Barlow et al., 1993). According to this hypothesis, the dark event rate should be strongly dependent on pH. However, observations indicating that thermal pigment activation depends on prior deprotonation of the Schiff base contradict this hypothesis (Firsov et al., 2002). Thus, new hypotheses have been developed to account for this contradiction. Ala-Laurila et al. (2004) suggested that the low-energy thermal activation pathway may not exist and is simply an analytical artifact. According to this hypothesis, activation by heat and by light may follow the same molecular route. Lórenz-Fonfría et al. (2010) proposed that thermodynamic and kinetic structural fluctuations of protein may facilitate retinal thermal isomerization of rhodopsin. However, a molecular theory that can present an adequate explanation for the discrete retinal dark events of vertebrate rhodopsin remains undeveloped.

Since natural lipid peroxidation is one of the main sources of UPEs (Nakano, 2005; Adam et al., 2005; Thar and Kühl, 2004; Catalá, 2006), and photoreceptors have the highest oxygen consumption and polyunsaturated fatty acid concentration in the body (Fliesler and Anderson, 1983), permanent UPEs should occur in the retina without any external photonic stimulation. Thus, Bókkon and Vimal's (2009) photochemical (*biophysical*) interpretation about the discrete dark noise of rods may be a possible explanation for the discrete retinal dark. However, to date, experimental evidence of spontaneous or visible light-induced ultraweak photon emissions in human or animal eyes has not been reported, but it is necessary to support the hypotheses set forth by Bókkon (2008) and colleagues (Bókkon and Vimal, 2009). Here, we present the first *in vitro* evidence of spontaneous and visible light induced UPEs from freshly isolated whole eye, lens, vitreous humor, and retina samples from rats.

2. Results

All isolated samples (*whole eye, lens, vitreous humor, and retina*) presented continuous, spontaneous, and basal photon emission without any excitation. In addition, after 300 s of basal recording followed by 30 min of adaptation to the dark, isolated rat whole eye, lens, vitreous humor, and retina were illuminated by monochromatic red, green, or blue light with 10, 20, or 40 s duration, respectively. As shown in Fig. 1, all samples presented continuous spontaneous biophoton emission and obvious delayed biophoton emission (Fig. 1A–C, E–G, I–K, M–O). All figures (Fig. 1A–C, E–G, I–K, M–O) show a similar