Introduction

The endocannabinoid (eCB) system, including eCBs, cannabinoid receptors, and enzymes regulating the level of eCBs, is present in the central nervous system of all mammals. In most cases, eCBs act as retrograde messengers binding to the widely distributed CB1 receptors (CB1Rs) to inhibit the neurotransmitter release at both excitatory and inhibitory synapses (Kreitzer & Regehr, 2001a, b; Ohno-Shosaku et al., 2001; Wilson & Nicoll, 2001; Freund et al., 2003; Yazulla, 2008). In addition to the two main cannabinoid receptors [CB1R and CB2 receptor (CB2R)], G protein-coupled receptor 55 (GPR55) has been suggested as a cannabinoid receptor since it is activated by anandamide, an endogenous cannabinoid, and tetrahydrocannabinol, an exogenous cannabinoid (Ryberg et al., 2007).

In fact, GPR55 is a receptor that is also responsive to other cannabinoids (Oka et al., 2007; Ryberg et al., 2007). While GPR55 is implicated in several physiological and pathophysiological functions (Liu et al., 2015), its role in the retina is as yet unknown. Early case reports in the 1970s suggested the existence of cannabis-mediated visual effects in humans, although the specific mechanisms or activation pathways are still not defined. Cannabis consumption can lead to an increase in glare recovery for low contrast stimuli (Adams et al., 1978), a reduction in Vernier and Snellen acuities (Kiplinger et al., 1971; Adams et al., 1975), blurred vision (Noyes et al., 1975), and changes in color discrimination and photosensitivity (Dawson et al., 1977). Given that most of these effects undoubtedly have a retinal component, recent investigations have focused on examining the endogenous cannabinoid system in the retina.

Cannabinoid receptor expression patterns are well documented in rodent and primate retinas (Yazulla et al., 1999; Straiker et al., 1999a, b; López et al., 2011; Zabour et al., 2011a, b; Cécyre et al., 2013), including vervet monkeys (Bouskila et al., 2012, 2013a, b).
The latter species were used as the animal model to study the distribution of cannabinoid receptors in the retina and revealed cell-type specific expression profiles of CB1R, CB2R, and GPR55. While CB1R is present in retinal neurons (Bouskila et al., 2012) and CB2R is strictly expressed in glial Müller cells (Bouskila et al., 2013a), GPR55 is exclusively found in rod photoreceptors (Bouskila et al., 2013b). A variety of anatomical and functional visual effects of cannabinoids have also been reported for every retinal cell type in both rodents and fish (Yazulla, 2008; Cécyre et al., 2013). However, these studies did not investigate GPR55 because of its recent and controversial deorphanization as a cannabinoid receptor (Henstridge et al., 2009). Despite neuroanatomical and physiological evidence showing that the eCB system can modulate the activity of retinal cells in mammals, very little is known about the specific contribution of GPR55 in retinal functioning. Only one study has reported that palmitoylethanolamide can increase the adaptation state of the retina (Kumar et al., 2012). This finding indirectly suggests that GPR55 plays a role in the eye.

Full-field electroretinography is widely used as a measure of retinal function (McCulloch et al., 2015). Electroretinogram (ERG) recordings represent the sum of electrical current over the entire retina, light-evoked responses in photoreceptors (cones and rods), neurons (horizontal cells, bipolar cells, amacrine cells, and ganglion cells), glial cells (Müller cells), and epithelial cells (Steinberg et al., 1985). Amplitude, latency, and overall shape of the ERG waves depend on the intensity of the flash and its duration, as well as the adaptation state of the retina. While scotopic ERGs represent the contribution of the rod system in dark-adapted conditions, photopic ERGs represent the contribution of the cone system in light-adapted conditions. In the present study, we investigated the effect of the intravitreal injection of lysophosphatidylglucoside (LPG), an agonist of GPR55 (Guy et al., 2015), or CID16020046 (CID), a selective GPR55 antagonist (Kargl et al., 2012), on the dark-adapted and light-adapted full-field ERGs in vervet monkeys. Given that GPR55 is exclusively expressed in rods (Bouskila et al., 2013b), we hypothesized that the modulatory effect of GPR55 would manifest itself only in the ERG curves obtained in scotopic conditions.

Material and methods

Animals

A total of 13 adult vervet monkeys (Chlorocebus sabaeus) were used for this study: four monkeys received an intravitreal injection of dimethyl sulfoxide (DMSO, the vehicle), four monkeys were monocularly injected with LPG (a selective agonist of GPR55), and five monkeys with CID (a potent and selective antagonist of GPR55) (Table 1). The animals were born and raised in enriched environments in the laboratories of the Behavioral Science Foundation (St-Kitts, West Indies). The animals were sedated with a combination of ketamine (10 mg/kg; Troy Laboratories, Glendenning, New South Wales, Australia) and xylazine (1 mg/kg; Lloyd Laboratories, Shenandoah, IA). This mixture has a minimal effect on the ERG (Nair et al., 2011). The depth of sedation was maintained at a sufficient level to prevent the animals from moving, but without causing respiratory depression. In this condition, the pupils were fully dilated to approximately 9 mm in diameter and the accommodation reflex was paralyzed with topical application of 1% tropicamide (Mydriacyl®) and 2.5% phenylephrine hydrochloride (Mydfrin®) (Alcon Laboratories, Fort Worth, TX). Intraocular pressure was monitored before and after the recording session by application tonometry (TonoPen XL®, Reichert Technologies, Depew, NY). The eyes were treated with 0.5% proparaconaine hydrochloride (Alcaine®, Alcon Laboratories, Fort Worth, TX) to anesthetize the cornea. The eyes were then protected by application of 2.5% methylcellulose (Gonak; Akorn, Inc., Buffalo Grove, IL) to prevent corneal drying. Body temperature was maintained between 36.5 and 38°C with a heating pad. Recording sessions lasted approximately 2 h for each animal, after which they were allowed to recover in their cage, and then returned to their prior naturalistic setting.

Drug administration

Intravitreal injections were performed in one eye only when the animals were in a sedated state. After inspection and examination of the eyes and lids, a topical anesthetic was applied over the injection site. The conjunctival and corneal surfaces were then moistened with methylcellulose (Moisture Eyes, Bausch Lomb, Rochester, NY) for 3 min. The eye was covered with sterile coatings and a Barraquer eye speculum (1.75 in., 10 mm wide small blades; Storz Ophthalmics, St Louis, MO) held the eyes open. The LPG was synthesized using the method described by Guy et al. (2015), where its specificity and selectivity were successfully tested on the spinal cord of mice and chicken. The antagonist CID was purchased from Tocris Bioscience (Cat. No. 4959, Ellsville, MO). With a 30G needle, 50 µL of drug solution (LPG or CID) was injected into the vitreous cavity, 2 mm

<table>
<thead>
<tr>
<th>Monkey ID</th>
<th>Sex</th>
<th>Weight (kg)</th>
<th>Monocular injection</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>2.925</td>
<td>DMSO</td>
<td>100%</td>
</tr>
<tr>
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<td>Female</td>
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<td>DMSO</td>
<td>100%</td>
</tr>
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<td>DMSO</td>
<td>100%</td>
</tr>
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<td>100%</td>
</tr>
<tr>
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<td>2.525</td>
<td>LPG</td>
<td>1 mg in 100% DMSO</td>
</tr>
<tr>
<td>6</td>
<td>Female</td>
<td>2.800</td>
<td>LPG</td>
<td>1 mg in 100% DMSO</td>
</tr>
<tr>
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<td>LPG</td>
<td>1 mg in 100% DMSO</td>
</tr>
<tr>
<td>8</td>
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<td>2.950</td>
<td>LPG</td>
<td>1 mg in 100% DMSO</td>
</tr>
<tr>
<td>9</td>
<td>Male</td>
<td>3.950</td>
<td>CID</td>
<td>1 mg in 100% DMSO</td>
</tr>
<tr>
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<td>2.825</td>
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</tr>
<tr>
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<tr>
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</tr>
<tr>
<td>13</td>
<td>Female</td>
<td>2.925</td>
<td>CID</td>
<td>1 mg in 100% DMSO</td>
</tr>
</tbody>
</table>
Role of GPR55 in monkey retinal function

prior to the corneal limbus. A similar volume (50 μL) of the vehicle DMSO was injected into the vitreous of one eye in four control animals in order to rule out the possibilities that the injection per se and/or change in intraocular pressure caused the effects attributed to the drugs. When the needle was removed, the injection site was compressed for a minute using a sterile cotton swab to avoid reflux. Before and after ERGs recordings, the fundi of both eyes were inspected with a PanOptic ophthalmoscope (Welch Allyn, New York, NY). The following week, topical antibiotic ointment was administered to the eye that had been injected, twice daily for 4 days.

Visual stimulation

Full-field stimulation was produced with an UTAS BigShot Ganzfeld light source (UTAS E-3000 electrophysiology equipment; LKC Technologies, Inc., Gaithersburg, MD) that was placed in front of the animal’s face. The intensity of the flashes ranged from 0.00025 to 790 (cd s)/m² delivered in full-field conditions, with a duration of <5 ms. Xenon flash luminance of 2.5 to 790 (cd s)/m² (0–25 dB in LKC units) was used for photopic conditions and Light-Emitting Diode (LED) flash luminance of 2.5 × 10⁻⁴ to 6 (cd s)/m² (−40 to 4 dB in LKC units) for scotopic conditions. Between stimuli, delay intervals of at least 15 s were implemented at high flash intensities when the eyes were dark-adapted. In light-adapted conditions, a steady background-adapting field (30 cd/m²) was maintained inside the Ganzfeld to continually saturate the rod system. Flash intensities and background luminance were calibrated using a research radiometer (IL1700 Photometer, International Light Inc., Newburyport, MA) with a SED033 detector placed at 36 cm from the source (at the same location as the cyclopean eye).

ERG recording

All experimental protocols followed the guidelines of the ISCEV (Marmor et al., 2009; Boussika et al., 2014; McCulloch et al., 2015). ERG recordings and signal processing ERGs were carried out in the morning. The recorded eye was covered with a corneal contact lens electrode (Jet electrodes, Diagnosys LLC, Lowell, MA) moistened with 1% carboxymethylcellulose sodium (Refresh Celluvisc, Allergan Inc., Markham, ON). The electrode was equipped with four small posts that kept the eyelid open. Reference and ground gold disc electrodes (Jet electrodes, Diagnosys LLC, Lowell, MA) were kept in place with adhesive paste (Ten20 conductive EEG paste, Kappa Medical, Prescott, AZ) at the external canthi and forehead, respectively. Responses were amplified 10,000 times and filtered with a band pass from 1 to 500 Hz except for the oscillatory potentials, which were extracted with the LKC software and with a band pass from 75 to 500 Hz. Each trace included a 20 ms pre-stimulus baseline. The recording protocol for assessing the effect of the drugs is summarized in Figure 1.

Statistical analysis

For the waveform analysis, the amplitude of the a-wave was measured from the baseline to the trough of the a-wave. The amplitude of the b-wave was measured from the trough of the a-wave to the peak of the b-wave. The latency was defined from the onset of the flash to the trough (a-wave) or peak (b-wave). If these values fell within the acceptable time window (a-wave: 10–50 ms from flash, b-wave: 50–150 ms from flash), and they were within 3 s.d. above or below the mean, then they were saved. A repeated measure analysis of variance (ANOVA) was used to determine if the values extracted from the ERG curves in the DMSO-injected group were different from the LPG-injected group or the CID-injected group. Each mixed model ANOVA included one repeated measures factor (intensity of the flash) and one between group factors (drug: DMSO, LPG, or CID).

Results

No changes in the fundus of the eyes were observed after the intravitreal injection in any of the animals. There were also no differences in the intraocular pressure (before: 11 ± 3 mm Hg; after: 11 ± 4 mm Hg) or the pupil size (before: 8.8 ± 0.3 mm; after: 8.8 ± 0.3 mm).

LPG increases the scotopic b-wave amplitude

The effect of LPG on the scotopic ERG was comparable across monkeys. Fig. 2A illustrates the average ERG waveform in dark-adapted conditions following the intravitreal injection of DMSO, LPG, or CID at 13 stimulus intensities. Each curve was generated from a simple mean across monkeys. As can be seen in the traces, the intravitreal injection of LPG increases the amplitude of the scotopic ERG. This effect was not significant for the a-wave amplitude (P = 0.972, Fig. 2B), but was significant for the b-wave amplitude (P = 0.050, Fig. 2C). On average, LPG caused the scotopic b-wave amplitude to increase by 36% relative to the control (DMSO).

In addition, the effect was significant at several specific flash intensities [−2.6 log (cd s)/m², P = 0.010; −2.2 log (cd s)/m², P = 0.007; −2.0 log (cd s)/m², P = 0.012; −1.0 log (cd s)/m², P = 0.015; −0.6 log (cd s)/m², P = 0.028] and marginally significant at other flash intensities [0 log (cd s)/m², P = 0.077; 1.4 log (cd s)/m², P = 0.074]. At the rod standard flash [−2.2 log (cd s)/m²], a flash intensity that corresponds to rod-driven responses, the increase caused by the injection of LPG was 52 ± 18 μV (52% relative to DMSO). At the combined rod-cone standard flash [0.6 log (cd s)/m²], a flash intensity that corresponds to mixed-rod-cone responses, LPG lead to an increase of 56 ± 40 μV (22% relative to DMSO). The latencies of LPG were comparable to DMSO [no significant difference for a-wave latency (P = 0.915, Fig. 2D) or the b-wave latency (P = 0.413, Fig. 2E)].
Fig. 2. Increase of the scotopic b-wave amplitude after intravitreal injection of LPG and decrease after CID. (A) Mean scotopic ERG responses after intravitreal injection of DMSO (black), LPG (blue), or CID (red) are shown for 13 flash intensities. On the one hand, the intravitreal injection of LPG, a GPR55 agonist, significantly increased the b-wave amplitudes. On the other hand, an overall significant decrease in the b-wave amplitude is observed after CID injection. (B–E) Averaged ERG amplitude and latency values are plotted as a function of flash intensities following injection. Error bars represent ±s.e.m. The * indicates a significant overall difference ($P < 0.05$) of CID and DMSO, or LPG and DMSO.
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Fig. 3. No significant changes in the photopic ERG responses after intravitreal injection of LPG or CID. (A) Mean photopic ERG responses after intravitreal injection of DMSO (black), LPG (blue), or CID (red) across 12 flash intensities are shown. (B–E) Averaged ERG amplitude and latency values plotted as a function of flash intensities following injection of DMSO (black), LPG (blue), or CID (red). Error bars represent ± s.e.m.
CID decreases the scotopic b-wave amplitude

The effect of CID on the scotopic ERG was comparable across monkeys. Fig. 2A illustrates the average ERG waveform in scotopic conditions. The most apparent feature of these curves is that the intravitreal injection of CID decreased the amplitude of the scotopic ERG. This effect was not significant for the a-wave amplitude (P = 0.197, Fig. 2B), but was significant for the b-wave amplitude (P = 0.004, Fig. 2C). CID had no significant effect on the latency for the a-wave (P = 0.113, Fig. 2D) or the b-wave (P = 0.089, Fig. 2E). The average decrease of the scotopic b-wave amplitude was 66%.

In addition, the effect of CID on the amplitude of the b-wave was significant at every individual flash intensity [−3.6 log (cd s)/m², P = 0.003; −3.4 log (cd s)/m², P = 0.003; −3.2 log (cd s)/m², P = 0.002; −2.8 log (cd s)/m², P = 0.018; −2.6 log (cd s)/m², P = 0.003; −2.2 log (cd s)/m², P = 0.001; −2.0 log (cd s)/m², P = 0.006; −1.0 log (cd s)/m², P = 0.027; −0.6 log (cd s)/m², P = 0.024; 0 log (cd s)/m², P = 0.016; 0.4 log (cd s)/m², P = 0.007; 0.6 log (cd s)/m², P = 0.002; 1.4 log (cd s)/m², P = 0.004]. At the rod standard flash [−2.2 log (cd s)/m²], CID decreased the b-wave amplitude by 66 ± 15 μV (67%) relative to DMSO. At the combined rod-cone standard flash [0.6 log (cd s)/m²], CID decreased the b-wave amplitude by 153 ± 37 μV (59%) relative to DMSO.

The oscillatory potentials are not affected by either compound

A one-way ANOVA was performed on the summed amplitude of the oscillatory potentials at the standard flash [optimal intensity of 0.6 log (cd s)/m²; Beci, 2001; Bouskila et al., 2014]. The results show that there was no significant difference between the vehicle and the agonist for the amplitude (DMSO, $\bar{\tau} = 61.9 \pm 14.4 \mu V$; LPG, $\bar{\tau} = 68.9 \pm 11.3 \mu V; P = 0.674$) or latency (DMSO, $\bar{\tau} = 21.0 \pm 0.6$ ms; LPG, $\bar{\tau} = 20.5 \pm 0.4$ ms; $P = 0.724$). A non-significant effect was also found between the vehicle and the antagonist for the amplitude (CID, $\bar{\tau} = 36.0 \pm 7.9 \mu V; P = 0.122$) and the latency (CID, $\bar{\tau} = 18.8 \pm 1.2$ ms; $P = 0.123$).

LPG has no significant effect on the photopic ERGs

The effects of LPG on the photopic ERG were comparable across monkeys. Fig. 3A illustrates the average ERG waveform across monkeys in light-adapted conditions following intravitreal injection of DMSO, LPG or CID for 12 stimulus intensities. There were no significant effects of LPG for a-wave amplitude ($P = 0.817$, Fig. 3B), b-wave amplitude ($P = 0.756$, Fig. 3C), a-wave latency ($P = 0.942$, Fig. 3D), or b-wave latency ($P = 0.727$, Fig. 3E).

CID has no significant effect on the photopic ERGs

The effects of CID and DMSO on the photopic ERG were comparable across monkeys, see averages plotted in Fig. 3A. There was no statistically significant difference between CID and DMSO: a-wave amplitude ($P = 0.321$, Fig. 3B), b-wave amplitude ($P = 0.067$, Fig. 3C). CID also had no significant effect on the a-wave latency ($P = 0.381$, Fig. 3D) or the b-wave latency ($P = 0.093$, Fig. 3E).

Discussion

This study investigated the functional consequences of monocular intravitreal injections of potent and selective GPR55 agonist (LPG) or antagonist (CID) in monkeys. Using electroretinographic recordings, we showed that under scotopic conditions, the administration of LPG significantly increased the amplitude of b-wave responses while CID caused them to decrease. The scotopic latencies were not affected by either compound, and neither were the amplitudes or latencies recorded under photopic conditions. This is the first evidence that GPR55 is only involved in scotopic vision since its activation by LPG, and its blockade by CID, modulated the dim-light retinal responses driven by the rod pathway. The strength of this result is visible in Fig. 4, which depicts the relationship between the relative amplitudes of the b- and a-wave (Perlman, 1983). This figure illustrates scotopic retinal function, and its modulation by either blocking GPR55 (impaired retinal function, e.g., nyctalopia) or by activating it (increased retinal function, e.g., hyper-scotopia). The functional effects reported here are in line with the anatomical localization of GPR55 in rod photoreceptors and confirms its purported role in scotopic vision.

GPR55 belongs to the Class A rhodopsin-like family of G protein-coupled receptors. Expression of GPR55 in the retina is limited to rod inner segments (Bouskila et al., 2013b), which is implicated in the generation of the scotopic ERG b-wave (Robson & Frishman, 1995; Tian & Slaughter, 1995), although this is somewhat debated (Miller & Dowling, 1970; Wen & Oakley, 1990). Given the expression pattern of GPR55 (Fig. 5A), we hypothesized that antagonizing this receptor in the retina would lead to a decrease in the scotopic ERG b-wave. This is indeed what we found, which can be explained by GPR55’s influence on the glutamate pathway (Sylantiev et al., 2013; Bouskila et al., 2013b). We speculate that the presynaptic GPR55 in rods is coupled to Gα13, and may have a role in the regulation of the postsynaptic mGlur6-signaling pathway in rod ON-bipolar cells. In normal conditions of dark adaptation, a large quantity of glutamate is released from rod photoreceptor terminals.
and binds to the metabotropic glutamate receptor mGluR6 located at the tip of ON-bipolar cell dendrites (Sampath & Rieke, 2004). The activation of GPR55 by LPG on rods coupled to G\(\alpha_{13}\), stimulates RhoA, Rho-associated protein kinase (ROCK), Phospholipase C (PLC) and opens Na\(^+\)/Ca\(^{++}\) channels resulting in membrane depolarization (Bouskila et al., 2013b) and hence an increased scotopic b-wave (Fig. 5B). Following the blockade of GPR55 with CID, the activation of G\(\alpha_{13}\), RhoA, ROCK, and PLC is consequently lowered resulting in the closing of Na\(^+\)/Ca\(^{++}\) channels, and hence a decreased scotopic ERG b-wave in the CID-treated eyes. The scotopic waveforms are taken from Fig. 2A at the rod standard flash, [-2.2 log (cd s)/m\(^2\)]. LPG, lysophosphatidylglucoside; R, rods; RBC, rod bipolar cells. Scale bar in (A): 30 \(\mu\)m and in (B) and (C): amplitude (vertical axis), 100 \(\mu\)V; latency (horizontal axis), 20 ms.

Night blindness (e.g., nyctalopia), a condition where it is nearly impossible to see in low light, is a symptom of many eye diseases like retinitis pigmentosa, Oguchi disease, and congenital stationary night blindness, which specifically targets the rods (Marc et al., 2003). Given the present findings, implication of GPR55 in scotopic vision might therefore be exploited as a pharmacological target in the treatment of retinal diseases that include symptoms of night blindness.

Acknowledgments

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References

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