Sildenafil Acutely Decreases Visual Responses in ON and OFF Retinal Ganglion Cells

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PURPOSE. Sildenafil (Viagra), a cGMP-specific phosphodiesterase type 5 inhibitor, is widely used for the treatment of erectile dysfunction and pulmonary hypertension. Clinical studies have reported transient visual impairments in patients after single-dose sildenafil use, suggesting neural involvement in several retinal layers, and also, possibly, retinal ganglion cells (RGCs), which provide the unique output of visual information to the brain. However, the effect of sildenafil on the RGC light responses is poorly understood. We therefore evaluated its effect on RGC spiking activity.

METHODS. We measured spontaneous and light-induced RGC spiking activity in Long-Evans rat ex vivo retinas by using the multielectrode array technique. Sildenafil citrate (0.3–30 μM) was applied to retinal preparations under continuous perfusion, during 10 to 60 minutes, followed by sildenafil washout.

RESULTS. A high concentration (30 μM) of sildenafil decreased the magnitudes of both ON- and OFF-type RGC light responses, to 26.3% ± 17% and 18.3% ± 7%, respectively, of the initial value, in a reversible and concentration-dependent fashion, while in 50% of RGCs all light responses were completely suppressed. Sildenafil also greatly increased the latency of both types of light responses. In this study, we provided evidence that extended exposure to both sildenafil and repeated light stimulation potentiates drug effects and delays recovery.

CONCLUSIONS. We found transient and concentration-dependent alterations of light responses at the RGC level after sildenafil exposure that are relevant for a better understanding of the acute visual effects of administration of this compound in humans.

Keywords: sildenafil, retinal ganglion cell, visual stimulus, electrophysiology

Sildenafil is a phosphodiesterase (PDE) type 5 inhibitor, which is widely used for both the treatment of erectile dysfunction1 and of pulmonary arterial hypertension.2 Patients with erectile dysfunction have experienced transient and mild impairments of color discrimination, which occur at the peak of the drug action.3 In addition, sildenafil decreases visual performance, particularly in terms of temporal response properties, in S-cone-isolating conditions;4 in rare cases, transient blindness has been reported.5 It has also been suggested that this drug may be a possible, but not yet confirmed, cause of anterior ischemic optic neuropathy.6 However, other studies have reported no visual toxic effects in either human patients or laboratory animals, even after long periods of daily drug use.7–9

The effect of the drug on retinal function has been investigated in various electrophysiological studies. In vivo electroretinogram (ERG) recordings show decreases in both a- and b-wave amplitudes in sildenafil-treated patients,10 or, alternatively, an increase in the scotopic ERG responses, but a decrease in the photopic responses is observed.11 Gabrielli and colleagues12 report an increase in the Naka-Rushton equation V_max parameter, suggesting higher rod response after sildenafil ingestion. A more consistent observation, among all human studies, is that sildenafil increases the latencies of the a- and b-wave.11,13,14 Ex vivo experiments have also shown some contradictory results. Sildenafil has been shown to increase ERG amplitudes in the rat retina,15 whereas it decreases ERG amplitudes while increasing their latencies in bovine and human retinas.16,17

These effects of sildenafil on retinal function may be mechanistically related to the inhibition of PDE5. This is expressed in retinal cells, including human retinal ganglion cells (RGCs), although its physiological role in these cells remains to be investigated.18 However, in addition to its effect on PDE5, sildenafil can also inhibit other PDEs, including PDE6, which controls the phototransduction cascade in photoreceptors.19,20 Sildenafil appears almost as potent on cone PDE6 as on PDE5, while it seems slightly less potent on rod PDE6.21,22 The functional relevance of blocking photoreceptor PDE6s has been demonstrated after rat retinal explant incubation with...
Sildenafil Decreases ON and OFF RGC Responses

**Sildenafil Specifically Inhibits PDE5 and PDE6.** PDE5 and PDE6 are primarily found in amacrine cells and some RGCs. However, cholinergic amacrine cells, and rods are detected in the retina, such as PDE4s in RGCs, bipolar cells, cholinergic amacrine cells, and rods. PDE1 in bipolar cells; and PDE9 in amacrine cells and some RGCs. However, sildenafil has a very low affinity for PDE1, PDE4, and PDE9, and primarily inhibits PDE5 and PDE6.

These previous studies have reported conflicting results on the effect of sildenafil on retinal function. We report the first analysis of the retinal output signal at the RGC level in the presence of sildenafil to help understand the effect of different concentrations of sildenafil on the principal characteristics of light-induced RGC responses (magnitude and latency) and their spontaneous firing rate.

**Materials and Methods**

**Ethical Approval**

Experiments were conducted in accordance with the guidelines on the ethical use of animals from the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research and the European Community Council Directive (86/609/EEC).

**Ex Vivo Multielectrode Array (MEA) Recordings**

Twenty-two Long Evans rats, 8 weeks old, were obtained from Janvier Labs (Le Genest Saint Isle, France). Animals were housed with a 12-hour dark/light cycle, and food (standard diet) and water were available ad libitum. They were killed by CO2 inhalation, followed by quick cervical dislocation under dim red light; both eyes were isolated and placed in oxygenated Ames’ medium (Sigma-Aldrich Corp., St. Louis, MO, USA) at room temperature. During retinal dissection, we used Ames’ medium equilibrated with 95% O2 and 5% CO2 at pH 7.4, taken from a perfusion system, with eyecups placed in a Petri dish under dim red light. Square pieces of retina (1–2 mm²) were placed into the recording chamber, with the RGC layer facing a MEA60 biochip electrode array (60 titanium nitride electrodes, each of 10-µm diameter, arranged in 8 x 8 layout with 100-µm interelectrode spacing [Multi Channel Systems (MCS), GmbH, Reutlingen, Germany]). The retinal pieces were covered with a 2.5- to 5-mm² square piece of translucent polycarbonate membrane, 0.4-µm pore size (Corning, Inc., Corning, New York, USA). Retinas were held in place with a U-shaped platinum ring and a nylon mesh. During recording sessions, retinas were bathed in Ames’ medium equilibrated with 95% O2 and 5% CO2 at pH 7.4. Retinas were maintained at 34°C to 37°C and perfused continuously at a rate of 1.3 mL/min. To obtain stable recordings, the sessions started 30 minutes after the retina was placed in the MEA recording chamber. Sildenafil citrate (Sigma-Aldrich Corp.) was bath-applied into the perfusion system at concentrations ranging from 0.3 to 30 μM. Washout periods of 10 minutes were included, except for the 0.3 μM sildenafil and the nondrug control cases, since, for these two conditions, no significant changes in spiking activity were observed for the main parameters analyzed (spontaneous activity, initial burst response, and latency) after 10 minutes of exposure. The effects of all concentrations were assessed in at least three independent experiments.

**Stimulation and Analysis**

Experiments were conducted with an MEA60 setup (MCS). Analog extracellular neuronal signals from 60 channels were AC amplified (×1000–×12000), band-pass filtered (200–3000 Hz), sampled at 20 to 30 kHz and saved in a PC-compatible computer for subsequent off-line analysis. Retinal ganglion cell spiking activity was monitored during experimental sessions by using MC_Rack software (MCS). Light-induced responses were evaluated under conditions of complete darkness. White light stimulation blocks produced from light-emitting diodes (LEDs) driven by a stimulus generator STG-1008 (MCS) were applied to eliciting light responses in the RGCs. The LEDs were positioned 5 mm below the transparent MEA and used to generate full-field stimuli in the photopic range (5.0 cd/m²). The stimulus consisted of 10 consecutive stimulus blocks, each with 5-second light followed by 10-second dark each. The stimulus was applied periodically (every 10 minutes) and the light responses recorded. This method of light stimulation was chosen on the basis of efficiency of light responses (particularly, strength and number of responsive cells) in our previous MEA recordings performed on isolated retina of rodent species. All recordings were subsequently submitted to off-line spike sorting and analysis with Spike2 (CED Co., Cambridge, UK). Waveforms were separated by using a combination of template matching and cluster cutting based on principal component analysis of the waveforms. The spontaneous mean firing rate was calculated for each RGC over a time window of 30 seconds. To detect changes in spontaneous activity induced by light, the rater and peristimulus time histograms (PSTHs) were generated from 10 stimulus blocks using different bin widths. The onset of ON- and OFF-type RGC responses was defined as an increase in spike number greater than 2 SDs of the prestimulus frequency over at least three consecutive 50-ms bins. The initial burst responses to both light onset (ON-type RGCs) and dark onset (OFF-type RGCs) were quantified over a 50 ms bin. The mean spiking rates over the subsequent 1- and 5-second epochs after light onset (ON-type RGCs) or dark onset (OFF-type RGCs) were also quantified to assess additional changes in response parameters after the initial burst. Most RGC light responses were classified as transient, with an initial burst response to light or dark onset followed by a rapid decrease in spiking activity. Latency was defined as the time delay between light or dark onset (ON- or OFF-type RGCs, respectively) and the light response as defined above, when aligned in raster plots for nine consecutive light stimuli.

**Statistical Analysis**

All parameters analyzed in this study were normalized to the baseline value obtained in the same RGC before drug exposure. Statistical analysis was performed with Prism 5 (GraphPad, La Jolla, CA, USA) using Kruskal-Wallis one-way analysis of variance followed by Dunn’s posttest. *P* values less than 0.05 were taken as significant. All values are presented as mean ± SEM.

**Results**

**Sildenafil at High Concentration Acutely Increases RGC Spiking Activity and Abolishes Light Responses**

ON- or OFF-type RGC light responses were recorded on a MEA upon a series of 10 consecutive stimulus blocks consisting of 5-second light followed by 10-second dark. We found a rapid, partial, or complete elimination of RGC light responses in both ON- and OFF-types 10 minutes after application of a high sildenafil concentration (50 μM; Figs. 1A–D). This confirms the hypothesis that sildenafil can affect retinal network function by...
reducing the light-evoked firing in RGCs. However, we noticed that, among the 10 consecutive stimulus blocks, the responses of ON-type RGCs specific to the first block appeared enlarged in both light and dark periods (Fig. 2A). This atypical ON-response was not observed in the following ON-responses, which were heavily suppressed (Figs. 1A, 1B). To compare to previous studies that used intermittent light flashes with long periods in darkness, we analyzed RGC light response to the first stimulus block (Fig. 2B). However, this atypical, and intriguing, ON-response pattern was only observed at 30 μM sildenafil. Considering the spontaneous activity in the dark, application of sildenafil (0.3–30 μM) for 10 minutes did not induce a statistically significant increase either in ON- or OFF-type light response, although at (and only at) the highest (30 μM) concentration, some cells exhibited a slight increase in spontaneous activity (Figs. 3A, 3B).

Figure 1. Effects of high concentration of sildenafil on RGC light responses. Examples of RGC light responses before (baseline) and after exposure to 30 μM sildenafil for 10 minutes. Series of 10 consecutive stimulus blocks, each consisting of 5 seconds of light period followed by 10 second dark, were delivered to ex vivo retinas. (A) Raw recordings for five consecutive stimulus blocks are shown for an ON-transient RGC. Note the enlarged response to the first stimulus block. (B) Peristimulus time histograms (PSTHs) and raster plots for ON-transient RGC responses are shown for five consecutive stimulus blocks. After 30-μM sildenafil application, RGC responses are acutely reduced or abolished. Note that raster plots, representing spiking events, cease to be aligned with light period of stimulus blocks. (C) Raw recording of an OFF-transient RGC to the light offset of a stimulus block is shown. (D) The PSTHs and raster plots of OFF-transient RGC responses are shown for five consecutive stimulus blocks. After 30-μM sildenafil application, RGC responses are acutely reduced or abolished. White rectangles indicate duration of light periods.
Sildenafil Affects the RGC Light Responses in a Concentration-Dependent Manner

We applied increasing sildenafil concentrations from 1 to 30 μM to further evaluate concentration-dependent behavior on the sildenafil effects on light responses. We always excluded the first stimulus block from the measurements for quantification, since this response included that of a nonadapted retina, where activity from neighboring RGCs introduced extra noise in individual recording electrodes. In ON-type RGCs the application (10 minutes) of sildenafil induced a concentration-dependent decrease in the magnitude of the light response (Fig. 4). Such an effect was observed for both the initial burst response to light onset (Fig. 4A), and the subsequent 1- and 5-second epochs (Figs. 4B, 4C). The highest sildenafil concentration (30 μM) completely suppressed the ON- and OFF-type light responses in 50% of RGCs. When light responses were detected for 30 μM, they were strongly reduced (Figs. 4A–C).

After sildenafil application, we perfused retinas with a drug-free Ames’ medium for up to 60 minutes to assess the reversibility of sildenafil effects on ON- and OFF-type responses. We also recorded the response to 10 consecutive stimulus blocks every 10 minutes in order to periodically evaluate the RGC light response over the entire protocol. After 60 minutes of washout, although a partial recovery (to 89.8% ± 19% for 10 μM and to 60.8% ± 9% of initial response for 30 μM) in response magnitude was seen for the initial burst (Fig. 4A), it was hardly found, notably for the later 5-second epoch (Fig. 4C); this may indicate residual alterations of retinal response for long periods after cessation of drug exposure. The latency of ON-response was also quantified, and we found that this principal parameter of light responses was greatly increased in a similar concentration-dependent fashion to the response magnitude (Fig. 4D). For 10- and 30-μM sildenafil concentrations, the increase in latency of ON-responses reached 272.9% ± 55% and 387.4% ± 106% of the initial response, respectively. On average, these percentages correspond to an increase from 65 ms on initial response to 193 ms after 10 μM sildenafil, and from 66 ms on initial response to 248 ms after 30 μM sildenafil. However, in contrast to the ON-response magnitudes, their latencies were mainly recovered, although not completely, after 60 minutes of washout. For a 10-μM sildenafil concentration, the ON-response latency partially recovered to 143% ± 7%, while for 30-μM concentration, the

**Figure 2.** Quantification of spiking activity for the first stimulus block of ON-type RGC responses following application of high concentrations of sildenafil. (A) Raw recordings for the first stimulus block for an ON-transient RGC before (baseline) and after exposure to 30 μM sildenafil for 10 minutes. The white rectangle indicates the duration of light period. (B) Quantification of spike counts for ON-transient RGCs after sildenafil application (3–30 μM) for 10 minutes. The graph indicates the number of spikes during the light period (white bars) and during the dark period (gray bars) of the first stimulus block. The spike count values were normalized to the measurements obtained during the baseline recordings. Sildenafil application induced an increase in spiking activity especially during dark period of stimulus block. n = 30 cells. *P < 0.05, compared to baseline.

**Figure 3.** Maintenance of the RGC spontaneous firing rate after sildenafil application. Effects of sildenafil application (0.3–30 μM, for 10 minutes) and a nondrug control on the RGC spontaneous spiking activity were evaluated. Retinal ganglion cells were divided in ON-type (A) and OFF-type (B). Spontaneous spiking rates were normalized to the baseline values obtained before drug exposure. Retinal ganglion cells exhibited a spontaneous baseline spiking rate of 4.6 ± 4.1 Hz for ON-type RGCs and 19.3 ± 8.6 Hz for OFF-type RGCs. Only at the highest sildenafil concentration (30 μM) did some RGCs show increased spontaneous spiking rate after sildenafil application, although no statistically significant difference was found. n = 63 (ON cells) and n = 85 (OFF cells).
recovery reached only 197.5% ± 22% of the initial response (Fig. 4D).

A similar pattern of concentration-dependent decrease of OFF-response magnitudes was found with the OFF-type RGCs (Figs. 5A–C). For the initial burst, the decrease here reached 41.8% ± 6% of the initial response for 10 and 30 μM, respectively (Fig. 5A). The 1- and 5-second response epochs also showed a reduction (Figs. 5B, 5C). Although some partial recovery to 70.4% ± 6% of response magnitude was seen for RGCs exposed to 10 μM sildenafil after 60 minutes of washout (Fig. 5A), no recovery was found with the higher (30 μM) concentration (Figs. 5A–C). The response latencies also increased markedly in a concentration-dependent fashion. These increases reached 311% ± 119% and 472.9% ± 67% of initial response for 10 and 30 μM, respectively. These percentages correspond to an average increase from 72 ms on initial response to 240 ms after 10 μM sildenafil, and from 74 ms on initial response to 384 ms after 30 μM sildenafil. A partial recovery was evident upon 60 minutes of washout (Fig. 5D), similarly to that seen with ON-type RGCs. These results, taken together, suggest that although light responses in ON- and OFF-type RGCs recover after removal of sildenafil, some of the response characteristics, such as magnitudes and latencies, may be slightly altered for longer periods.

**Potentiation of Sildenafil Effects With Extended Exposure**

The possibility of cases of sildenafil abuse cannot be discarded, in particular those arising from combination with illicit drugs, which may result in unpredictable pharmacokinetics even at therapeutic doses. Because of this, we attempted to evaluate how longer exposures to lower concentrations would further potentiate the sildenafil-elicited modifications on RGC light responses. The duration of drug application was therefore extended to 60 minutes, both for concentrations ranging from 0.3 to 3 μM sildenafil, and for the nondrug control; this was followed by washout. The response to 10 consecutive stimulus blocks was recorded every 10 minutes. Even concentrations as low as 0.3 and 1 μM produced a greater efficacy in ON-type RGCs, with response magnitude reduced to 83.8% ± 12% and 69.6% ± 10% of initial response, respectively (Fig. 6A). The sildenafil effect was enhanced with extended exposure for both the 1- and 5-second epochs (Figs. 6B, 6C). Note that 60 minutes of sildenafil washout was not sufficient to recover the initial response magnitude (Figs. 6A–C), suggesting a massive impregnation of the tissue, thus requiring longer recovery periods. In addition, it may be taken into account that the relatively high lipophilicity of sildenafil might result in slow washout. For response latencies of ON-type RGCs, the same pattern upon response magnitudes was found, with increased effect of sildenafil in latencies, with extended exposure (Fig. 6D).

Extended exposure (60 minutes) to sildenafil also potentiated the effects on response magnitudes with OFF-type RGCs (Figs. 7A–C). For the lowest (0.3 μM) concentration used, the initial burst response was not affected, even after 60 minutes of drug application, suggesting stronger resilience of OFF-type RGCs compared with ON-type RGCs under these conditions. However, the 5-second epoch (Fig. 7C), but not the 1-second one (Fig. 7B), was reduced to 68.2% ± 11% of initial response after 60 minutes of 0.3-μM sildenafil application. This may be explained by the natural decrease of spiking activity observed during the recording sessions, as can be seen for nondrug.
control. Nevertheless, we highlight the fact that the initial burst to the dark onset was preserved and more reliably indicates whether an OFF response is present. For concentrations of 1 and 3 μM sildenafil for 60 minutes, we found that the initial burst was reduced to 59.6% ± 3% and 33.4% ± 2%, respectively (Fig. 7A); the 1- and 5-second epochs (Figs. 7B, 7C) presented similar behavior. Although a small, but statistically significant, recovery could be found for initial burst for 1-μM sildenafil concentration within 60 minutes of washout (Fig. 7A), this effect was not clear for 3-μM sildenafil concentration.

We found increased values after extended sildenafil exposure to 60 minutes with response latencies of the OFF-type RGCs. These reached 157% ± 5% and 312.7% ± 13% for 1 and 3 μM, respectively, after 60 minutes of sildenafil exposure. This corresponds to an average change from 80 and 72 ms on initial response to 114 and 333 ms after 60 minutes of sildenafil exposure. A clear recovery of latencies was observed within 60 minutes of washout. For 1 and 3 μM, latencies returned to 120.8% ± 5% and 145.2% ± 6% of initial response (Fig. 7D). It must be noted that the recovery from the sildenafil effect was not so evident in ON-type RGCs, notably after 60 minutes of 3-μM sildenafil application.

Finally, we also evaluated the effect of extended sildenafil (1 and 3 μM) exposure on RGC spontaneous activity. Although no concentration-dependent effect was observed (Figs. 8A, 8B), the spontaneous activity of both ON- and OFF-type RGCs seems to be decreased after 60 minutes of washout, although a statistically significant difference was only found for the OFF-type RGCs (Fig. 8B). This may reflect a prolonged effect of sildenafil potentiated by light stimulation.

**DISCUSSION**

Our results demonstrated that sildenafil has a significant and concentration-dependent effect on the retina and, in particular, on the RGCs, the output neurons of the retina. This illustrates the critical changes in principal characteristics of RGC light response, which include a concentration-dependent decrease in magnitude and an increase in latency of light responses. These were only partly restored during 60 minutes of washout. Even with low concentrations, which can be measured in human plasma (e.g.,1 μM) after sildenafil administration,31 the RGC light responses showed statistically significant decreases in response magnitudes and increases in latencies, particularly after prolonged drug exposure (60 minutes) and repetitive light stimulation. The recovery of light responses was incomplete, notably for ON RGCs, even after prolonged (60 minutes) washout, which, for other chemicals, traditionally applied to the retina, normally takes up to 15 minutes.28

Since the introduction of sildenafil as a treatment for erectile dysfunction, reports of sildenafil-induced visual alterations have emerged.3 Nonarthritic ischemic optic neuropathy has been reported in patients after sildenafil use, although a direct cause–effect has been difficult to demonstrate.52,53 Possible drug-induced modifications of RGC activity could

![Figure 5](https://example.com/figure5.png)

**Figure 5.** Concentration-dependent and reversible effects of sildenafil on OFF-type RGC light responses. Sildenafil application (1–30 μM) for 10 minutes in a concentration-dependent fashion reduces the magnitude of OFF-type RGC response to dark onset. This reduction was found at both initial burst (A) and at 1-second (B) and 5-second (C) epochs. The response latencies were also increased in a concentration-dependent manner (D). After 60 minutes of washout, although some partial recovery of response magnitude was seen (A), no recovery was found with increasing sildenafil concentrations (A–C). Similarly to ON-type RGCs, the response latencies showed a clear washout of sildenafil effect (D). All results were normalized to the baseline values obtained before drug exposure. n = 56 cells. *P < 0.05, **P < 0.01, compared to baseline; #P < 0.05, compared to sildenafil 10 minutes.
The effects of extended exposures to low concentrations of sildenafil on ON-type RGC light responses. Sildenafil (0.3–3 μM) application for 60 minutes induced a reduction of RGC responses to light onset more prominent than 10-minute application (A). For initial burst (A) and for 1-second (B) and 5-second (C) epochs, the reduction of RGC responses was higher than with sildenafil for 10 minutes. Also, the reversibility of sildenafil effect upon 60 minutes of washout was difficult after 60 minutes of drug exposure (A–C). For the lowest concentration (0.3 μM) and for the nondrug control, washout periods were not recorded. The response latencies exhibited increased values in a concentration-dependent manner after 60 minutes of sildenafil application (D), and although for 1 μM sildenafil there was a small but statistically significant recovery within 60 minutes of washout, the same was not observed for 3 μM sildenafil. All results were normalized to the baseline values before drug exposure. n = 55 cells. *P < 0.05, **P < 0.01, ***P < 0.001, compared to baseline; #P < 0.05, compared to sildenafil 60 minutes.

Contribute to an optic neuropathy. Thus, the reported transient episodes of blindness could result from the complete disappearance of some ON- and OFF-type RGC light responses, as we observed after exposure to high (30 μM) sildenafil concentrations (Fig. 1). Moderate impairments in color discrimination have also been reported in human volunteers after sildenafil administration. These latter transient alterations appear to correlate with the peak of sildenafil plasma concentration and are fully reversible. In a trial assessing ocular safety of sildenafil use for pulmonary arterial hypertension, transient adverse events, such as chromatopsia, photophobia, and visual brightness, have been reported with the highest dose of 80 mg sildenafil administered three times daily for 12 weeks; however, no permanent detrimental effect on visual function is found after such a chronic dosage of sildenafil. It can be suggested that these transient visual symptoms could result from the decreased or delayed RGC light responses, as has been found in this study. More specifically, the RGC response latencies are known to be key encoding components in the transmission of spatial structure of images to visual brain centers.

Studies on human and laboratory animals both yield contradictory data concerning acute sildenafil effects. Both increased and decreased light responses have been reported after sildenafil administration, particularly with ERG measurements. Such apparently inconsistent results could be attributed to the atypical initial increase in light response magnitude, followed by subsequent decreases. Barabás and colleagues have found increased ERG amplitude with 1 μM sildenafil in rat retina, while other authors, using bovine retinas, have reported decreased a- and b-wave amplitudes with sildenafil concentrations as low as 0.3 μM and 0.1 μM, respectively. In a recent study, an increased bipolar cell response is found 1 hour after sildenafil treatment in rd1/−/− mice, while this response is reduced after 2 days. Different doses and light stimulation paradigms are applied across these different studies, although some of them focus on only one sildenafil dose, such that the results are difficult to compare. Another complicating factor is the presence (in vivo) or absence (isolated retinas) of functional ocular vasculature and retinal pigment epithelium (RPE), as sildenafil affects ocular blood flow and increases cGMP accumulation in RPE, respectively. In the present study, we evaluated the effects, particularly on RGC light responses, of sildenafil application in an isolated rat retinal preparation without RPE; to study this question, which has never previously been addressed, we used various drug concentrations, including low concentrations, which have been found in human plasma after sildenafil administration (1 μM). In our experiments, the light responses were modified, even at the lowest concentrations (0.3 and 1 μM), whereas the RGC spontaneous activity did not display a statistically significant difference even at the highest drug concentration (Fig. 3).

The attenuation of RGC light responses and extended time to peak/ latency found in this study (Figs. 4–7) are in agreement with previous reports showing decreased ERG amplitudes and increased implicit time in bovine and human retinas. Interestingly, we showed that extended exposure at lower concentrations, together with the repetitive light stimulation, potentiated the effectiveness of sildenafil-induced attenuation of light responses (Figs. 6, 7). In particular, the ON-type responses did not completely recover, even after extended periods of washout. The concentration of 1 μM is readily found
in human plasma after sildenafil use. Moreover, it is possible that some patients, particularly those with liver disease or renal dysfunction, take doses above the recommended therapeutic ones, reaching peak plasma concentration above 1 μM.

Although we did not find strong effects on RGC light responses at concentrations of 1 and 3 μM after 10 minutes of sildenafil exposure, extending the drug exposure to 60 minutes suppressed the light response magnitude and delayed laten-

FIGURE 7. Effects of extended exposures to low concentrations of sildenafil on OFF-type RGC light responses. Sildenafil (0.3–3 μM) application for 60 minutes induced a reduction of RGC responses to dark onset more prominent than for 10-minute application, at the level of initial burst (A), and at 1-second (B) and 5-second (C) epochs. Recovery from sildenafil effect upon 60 minutes of washout was seen for the initial burst (A), but no recovery was found for 1-second (B) and 5-second (C) epochs. For the lowest concentration (0.3 μM) and for the nondrug control, washout periods were not recorded. The response latencies exhibited increased values in a concentration-dependent manner after 60 minutes of sildenafil (D). However, a clear recovery of response latencies was found upon 60 minutes washout. All results were normalized to the baseline values before drug exposure. n = 46 cells. *P < 0.05, **P < 0.01, ***P < 0.001, compared to baseline; #P < 0.05, compared to sildenafil 60 minutes.

FIGURE 8. Effects of extended exposures to low concentrations of sildenafil on RGC spontaneous activity. Effect of sildenafil application (1 and 3 μM, 10–60 minutes) on the RGC spontaneous spiking activity was evaluated. Retinal ganglion cells were divided in ON-type (A) and OFF-type (B). Spontaneous spiking rates, when detected, were normalized to the baseline values obtained before drug exposure. For both ON- and OFF-type, RGCs exhibited a small decrease for 1 μM after 60 minutes of sildenafil exposure, though no concentration-dependent effect was observed. However, spontaneous activity of both ON- and OFF-type RGCs seems to be decreased after 60 minutes of washout, although statistically significant difference was found only for OFF-type RGCs (B). All results were normalized to the baseline values before drug exposure. *P < 0.05, compared to baseline. n = 34 (ON cells) and n = 59 (OFF cells).
cies, similarly to the effects seen for shorter sildenafil exposure (10 minutes) with higher concentrations.

There was an atypical increase in response amplitude in the first stimulus block when measuring RGC light responses upon the 30-μM sildenafil application (Fig. 2). However, the following responses to subsequent stimuli were greatly decreased. This transient sildenafil potentiation of the light response could result from the reported increase in photoreceptor sensitivity after exposure to this drug.12,15 The partial sildenafil inhibition of PDE6 could increase the photoreceptor cGMP intracellular concentration, thus changing the photoreceptor functional response dynamics. Accordingly, continuing PDE6 inhibition would lead to a progressive increase in photoreceptor cGMP, and a consequent constant photoreceptor depolarization.22,39 Such an excessive activation of cGMP-gated channels may generate risks for the cell viability, as we have previously reported with retinal explants.43 This transient potentiation of the RGC light responses would be consistent with enhanced PDE6 inhibition by sildenafil upon light stimulation. The transient potentiation of the ON responses could also result from a cGMP increase in ON bipolar cells, which has been reported to greatly enlarge the current suppressed by glutamate at the light onset.40 However, we note that sildenafil has very low affinity for PDEs present in bipolar cells.24,25,27

The intrinsically photosensitive RGCs, the only RGCs able to respond to light stimulus, represent a minority of the RGC population and are known to use a phototransduction cascade similar to rhabdomeric photoreceptors, which is not based in PDE6.41 On the basis of functional evidence, Contini and colleagues42 have suggested the presence of PDE6 in chicken RGCs; the authors have used zaprinast, which is also an inhibitor of PDE5. We therefore suggest that the effects we found on light-evoked RGC responses might result mainly from inhibition of PDE6 in photoreceptors by sildenafil, in agreement with the reduced and delayed a-wave found in ERG recordings in isolated bovine and human retinas.4,6,17,19 This, in turn, is expected to reduce both ON- and OFF-type RGC light responses.

In summary, we found that sildenafil attenuates RGC light responses in terms of magnitude and latencies in a concentration-dependent fashion. Moreover, these effects were potentiated by extended drug exposure and repeated light stimulation. To our knowledge, this is the first time the effects of sildenafil directly linked to RGC spiking activity have been evaluated. This study highlighted the acute effects of sildenafil on RGC light responses, which are the only inputs to brain visual centers. Transient losses of light responses, or at least magnitude decreases, warn against sildenafil abuses,30 which has been reported to greatly enlarge the current suppressed by glutamate at the light onset.40 However, we note that sildenafil has very low affinity for PDEs present in bipolar cells.24,25,27

References


Acknowledgments

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44. Liew KB, Loh GO, Tan YT, Peh KK. Simultaneous quantification of sildenafil and N-desmethyl sildenafil in human plasma by UFLC coupled with ESI-MS/MS and pharmacokinetic and bioequivalence studies in Malay population [published online ahead of print November 17, 2014]. Biomed Chromatogr. doi:10.1002/bmc.3578.