RESEARCH PAPER

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Dopamine D2 receptor blockade differentially affects the light-adapted turtle and frog electroretinogram

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The effects of dopamine D2-class receptor blockade by sulpiride on the electroretinographic (ERG) b-wave (ON response) and d-wave (OFF response) were investigated in light-adapted turtle (*Trachemys scripta elegans*) and frog (*Rana ridibunda*) eyecups. For turtle ERG, sulpiride (240 μM) produced an amplitude increase of the b- and d-waves, while the 40 μM and 120 μM of sulpiride were ineffective. Alternatively, for frog ERG, a well-developed and dose-dependent b- and d-wave amplitude decrease was obtained with 40 μM and 240 μM sulpiride. In both species, 240 μM sulpiride significantly affected the maximal voltage range of the ERG responses without altering their relative sensitivity (determined by the semi-saturation point). The absolute sensitivity of the ON and OFF responses (evaluated by threshold estimation) was not significantly altered for turtle ERG, but it was decreased for frog ERG. The time characteristics of the ERG responses were unchanged in both species. Our results show important differences between dopamine D2-class receptor-mediated pathways in turtle and frog retina (revealed by ERG).

Key words: dopamine, D2 receptors, electroretinogram, frog, turtle

INTRODUCTION

Dopamine is the major catecholamine in the vertebrate retina. All vertebrate species have retinal dopaminergic neurons identified as amacrine and/or interplexiform cells (for reviews: Popova 2014a, Witkovsky 2004). Dopamine released by these neurons can act on two classes of dopamine receptors: D1-class (including D1 and D5 type) and D2-class (including D2, D3 and D4 type). The activity of all retinal neurons can be modulated by dopamine because they all express D1- or D2-class receptors (for review: Popova 2014a). The significance of dopamine action, mediated by D1- or D2-class receptors, for global retinal function can be revealed by investigating changes of the electroretinogram (ERG) during manipulation of the two receptor classes. The most prominent ERG components in response to long lasting stimuli are the b-wave (in response to stimulus onset) and the d-wave (in response to stimulus offset). The primary neuronal generators of the b-wave are the depolarizing (ON) bipolar cells, while d-wave generation depends mainly on the activity of hyperpolarizing (OFF) bipolar cells (reviews: Frishman 2006, 2013). The effects of dopamine on ERG b- and d-waves, mediated by each dopamine receptor class (D1 or D2), have been investigated by using D1 or D2 receptor knockout animals or by application of specific dopamine receptor agonists and antagonists. Antagonists, as an application tool, have advantages over agonists because dopamine receptors undergo desensitization as a result of prolonged exposure to agonists (Barton et al. 1991, Gardner et al. 2001). In a previous study, we showed that isolated blockade of D1- and D2-class receptors had opposing effects on the intensity-response function of the cone-dominated b- and d-waves in frog ERG (Popova and Kupenova 2013, Popova 2014b). While D1 receptor blockade by 10 μM SCH 23390 enhanced ERG b- and d-wave amplitudes, D2 receptor blockade by 40 μM sulpiride suppressed amplitudes. We also demonstrated that combined D1 and D2 receptor block-

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ade has a suppressing effect on b-wave amplitude over the whole intensity range, while its effect on d-wave amplitude depends on stimulus intensity. According to these results, it appears that endogenous dopamine enhances the cone-dominated ON response (through action on D2-class receptors) and thus it can serve as a chemical messenger for light adaptation in frog retina. Contradictory results exist, however, concerning the D2-class receptor-mediated dopamine effects on light-adapted ERG in other species. While blockade of D2-class receptors enhanced b-wave amplitude in fish retina (Mora-Ferrer and Behrend 2004, Kim and Jung 2012), a decreased or unaltered b-wave amplitude was reported in both D4 and D2 receptor knockout mice (Jackson et al. 2012, Lavoie et al. 2014). Data concerning the effects of D2-class receptor blockade on the ERG OFF response are very limited. Most of the authors cited above used very brief light stimuli and thus the ON and OFF responses were fused in their ERG recordings. Mora-Ferrer and Behrend (2004), who used longer stimuli, reported a dampening and prolongation of the fish photopic OFF response under the influence of sulpiride. There is a question of whether, in conditions of light adaptation, the global effect of dopamine mediated through D2-class receptors differs among various species depending on retina type (rod-dominated, mixed rod-cone or cone-dominated). This needs to be clarified for both the ON and OFF responses.

In the present study, the effects of dopamine D2-class receptor blockade by sulpiride on the ON and OFF responses of the turtle ERG were investigated. Turtles were chosen because they have a cone-dominated retina and a well-expressed OFF component in their ERG. Dopaminergic neurons in turtles are morphologically well-characterized (Kolb et al. 1997), however their role, mediated by the D2-class receptors, in global retinal function is largely unknown. Therefore, in this work, we investigated the effects of different concentrations of sulpiride on the intensity-response function of the light-adapted turtle ERG b- and d-waves and compared them to results obtained from frog mixed-type retina in the present study, as well as from our previous studies (Popova and Kupenova 2013, Popova 2014b).

METHODS

Subjects and drug application

All experiments were carried out on turtle and frog eyecups. Turtles (*Trachemys scripta elegans*) and frogs (*Rana ridibunda*) of both sexes were supplied by a licensed supplier and were bred locally in the vivarium of the Medical University of Sofia. They were anesthetized with Tricaine (Sigma) dissolved in the bathing water to a concentration of 500 mg/l and then decapitated and pithed. All procedures performed in the study were approved by the Committee for ethics in scientific research of the Medical University of Sofia and the experiments were authorized by the Bulgarian Food Safety Agency.

The eyecup preparations were placed in a chamber where they were continuously superfused with Ringer solution (NaCl 110 mM, KCl 2.6 mM, $NaHCO_3$ 10 mM, $CaCl_2$ 2 mM, $MgCl_2$ 2 mM, Glucose 2 mM; HCl 0.5 mM to adjust pH to 7.8) at a rate of 1.6 -1.8 ml/min, temperature 18 – 20^0 C and supplied with moistened O_2 (for details see Kupenova et al. 2010, Popova 2014b). The D2-class receptors were blocked by sulpiride (Santa Cruz Biotechnology, Inc., Dallas, TX). Sulpiride was first dissolved in 10 mM HCl to a concentration of 2 mM and then a portion of this stock solution was dissolved in Ringer solution to achieve each final concentration as needed.

Experimental procedure and groups

The animals were dark-adapted for 24 h prior to the beginning of the experiments. Then the eyecups were prepared under dim red light. The eyecups were adapted to a white photopic background (150 W tungsten halogen lamp) with an intensity of 2.4×10^6 quanta s^{-1} μm^{-2} for 15 min. Then they were rhythmically stimulated with diffuse white light stimuli (150 W tungsten halogen lamp) with 5 s ON and 25 s OFF periods presented on the same background. The test stimulus intensity was changed over a range of 5 log units by means of neutral density filters (Esco Optics). The maximal stimulus intensity (log I=0) was 6×10^8 quanta s^{-1} μm^{-2} at the plane of the retina.

Two main groups of experiments were performed. In the first group (n=13 for turtles; n=16 for frogs), the effects of 40 μM sulpiride were followed for a period of 15–20 min by using light stimuli with constant intensity (log I=-2.5). This group was evaluated in order to test the dynamics of the sulpiride effects (Ringer solution in the control experiments). A concentration of 40 μM was chosen on the basis of our previous results indicating that it has marked effects on the dark-adapted frog ERG (Popova and Kupenova 2013). In the second group (n=34 for turtles; n=12 for frogs), stimuli with increasing intensity over a range of 5 log units were applied under the same background in order to investigate the V – log I function of the ERG waves. In each eyecup, two V – log I functions were obtained, which were separated by a 15 min adaptation period (without rhythmic stimulation). In the control experiments

both functions were obtained during a perfusion with Ringer solution. In the test experiments the first V – log I function was obtained during a Ringer solution perfusion and the second during a perfusion with sulpiride. Three concentrations of sulpiride—40 μM, 120 μM and 240 μM were tested in turtles. These concentrations cover the higher range of concentrations used in ERG experiments with lower vertebrate species (tiger salamander—10 μM, Perry and George 2007; goldfish—10 μM, Mora-Ferrer and Behrend 2004, 200 μM, Kim and Jung 2012). In frogs, only the highest concentration (240 μM) was tested and the results were compared with effects obtained from the lowest concentration tested in our previous study (Popova 2014b). The latter was done in order to exclude a nonspecific effect of application for this very high concentration.

ERG recording and data analysis

The ERGs were recorded by non-polarized Ag/AgCl electrodes at a bandpass of 0.1-1000 Hz and digitized at 2 kHz (Biopac system MP 150, Biopac Systems, Inc., 42 Aero Camino, Goleta, California 93117, USA, AcqKnowledge 4.3.1 software). B-wave amplitude was measured from the peak of the a-wave to the peak of the b-wave, while the d-wave was measured from the baseline to the peak of the wave. The peak amplitudes of the responses to stimuli of different stimulus intensities were used for V – log I function evaluation. The b-wave V – log I function was fitted to a generalized form of the Naka-Rushton equation: $V=V_{max} \times I^n / (I_\sigma^n + I^n)$, where *V*, amplitude of the ERG b-wave; V_{max}, b-wave maximal amplitude; *I*, stimulus intensity above the background; I_σ, stimulus intensity required to produce half-maximum amplitude; *n*, an exponent, related to the steepness of the V – log I function (Naka and Rushton 1966). The value of I_σ was used as an index of the response relative sensitivity, while the response absolute sensitivity was assessed by its threshold, determined by using 5 μV criterion response amplitude. The V - log I function of the d-wave was estimated by smoothing the experimental data using an inductive algorithm for smooth approximation of functions (Kupenova 2011). The threshold intensity (5 μV criterion response amplitude), V_{max} and I_t, producing 0.5 V_{max} (I_σ), were derived from the approximating curves. The time characteristics of the ERG waves were assessed by measuring their latency and implicit time. The latency was measured from stimulus onset (for b-wave) or offset (for d-wave) to the beginning of the wave, while their implicit time was measured from stimulus onset (for b-wave) or offset (for d-wave) to the peak of the wave.

For statistical evaluation of the data, paired Student's t-test, two-way ANOVA and two-way repeated measures ANOVA with Tukey *post hoc* test were used (OriginPro 18 software, OriginLab Corporation, Northhampton, MA). A p value<0.05 was considered significant.

RESULTS

Dynamics of the sulpiride effects

This experiment group was carried out in order to evaluate the time course of the blocker effects. ERG was first recorded with constant stimulus intensity (log I=-2.5) under control conditions during perfusion with Ringer solution for 10 min and then during perfusion with 40 μM sulpiride for another 15 min in turtle (n=7) and 20 min in frog (n=10) experiments. In the control experiments (n=6 for turtles; n=6 for frogs) the eyecups were perfused with Ringer solution throughout the whole time period.

Perfusion with 40 μM sulpiride caused no apparent changes in the turtle ERG waves. The b- and d-wave amplitudes obtained during the blocker application did not differ significantly from the corresponding values obtained in control experiments (two-way ANOVA, p>0.05) (Fig. 1A, B). The shape of the ERG waves also remained unchanged under the influence of sulpiride (Fig. 1C).

Perfusion with 40 μM sulpiride had a marked depressing effect on the b- and d-wave amplitude in frog ERG, which reached a plateau at the 10th minute from the beginning of blocker application (Fig. 1C, D). During the plateau period (from the 21st to 30th minute, timing from the beginning of the experiment) the amplitudes of the b- and d-waves were significantly smaller (two-way ANOVA, p<0.0001) than those obtained in the control experiments. No significant interaction between the blocker effect and time was found during that period (two-way ANOVA, p>0.05). This allowed us to obtain the V – log I function of the ERG responses in the second group of experiments during the constant effects of sulpiride. The b- and d-wave amplitudes recovered to a degree during reperfusion with Ringer solution (Fig. 1F). These results were very similar to our previous results, where the dynamics of 40 μM sulpiride effects were investigated in dark-adapted frog eyecups (Popova and Kupenova 2013).

One possible cause for the negative results obtained in turtles is that dopamine, through D2-class receptors, may modulate the ERG responses at stimulus intensities differing from the intensity used in this group of experiments. Thus, in the next group of experiments the effects of 40 μM sulpiride on ERG b- and d-waves were investigated over a wide range of stimulus intensities.

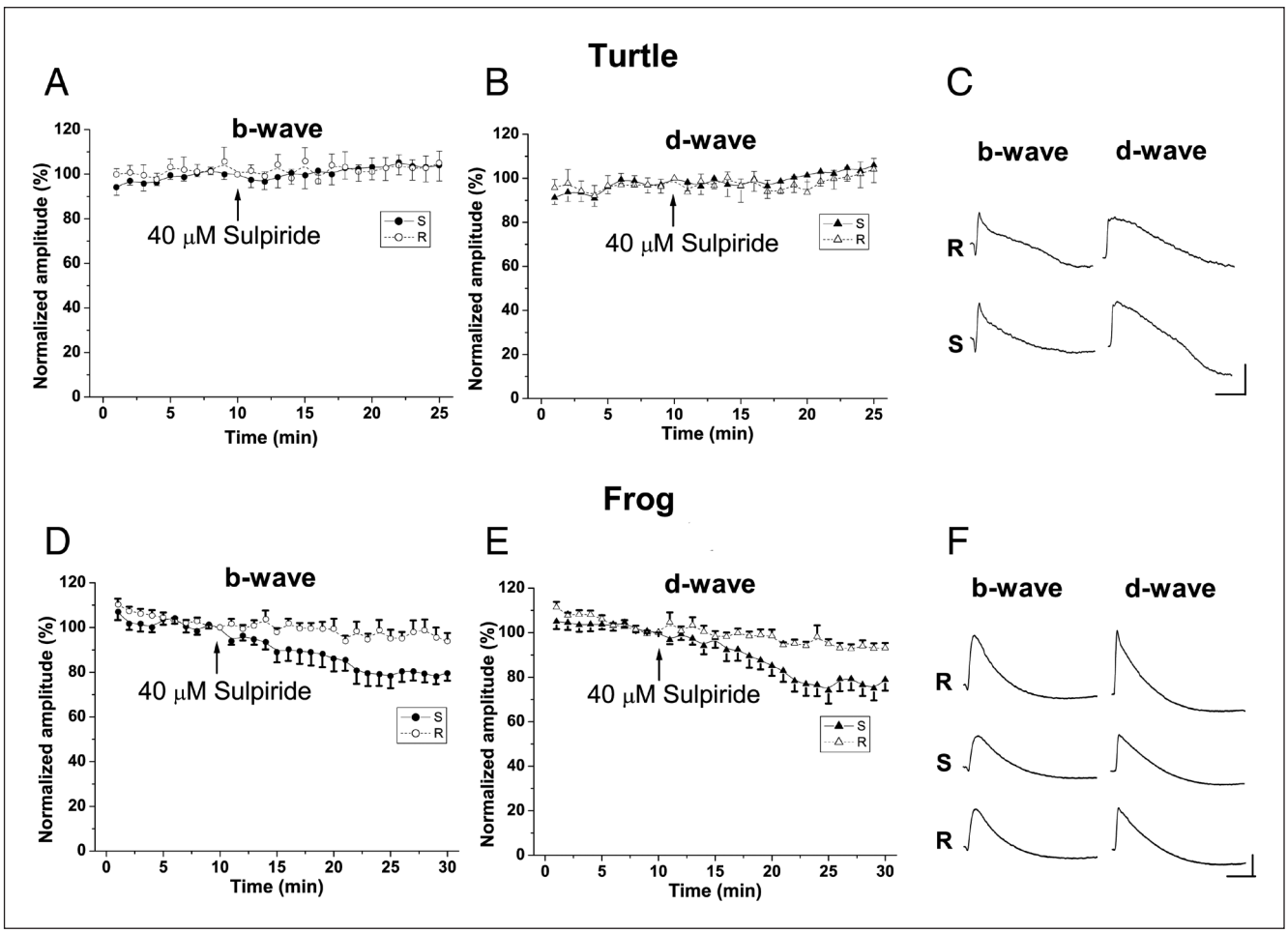

Fig. 1. (A), (B) Time course of sulpiride's effect on the amplitude of b- and d-waves, obtained with log I=-2.5 in light-adapted turtle eyecups. Results of both control experiments (open symbols; n=6) and test experiments (filled symbols; n=7) are represented. The amplitudes of the ERG waves were normalized to the values obtained just prior to blocker application (or at the corresponding time in the control experiments). The time point where the perfusion was switched to 40 μM sulpiride is indicated by an arrow. Mean values ± SEM are shown. (C) Original turtle ERG records (b- and d-waves), obtained during perfusion with Ringer solution in the control period (top traces) and 40 μM sulpiride (bottom traces). Calibration: time – 0.25 s, amplitude – 50 μV. (D), (E) Time course of sulpiride's effect on the amplitude of b- and d-waves, obtained with log I=-2.5 in light-adapted frog eyecups. Results of both control experiments (open symbols; n=6) and test experiments (filled symbols; n=10) are represented. The amplitudes of the ERG waves were normalized to the values obtained just prior to blocker application (or at the corresponding time in the control experiments). The time point where the perfusion was switched to 40 μM sulpiride is indicated by an arrow. Mean values ± SEM are shown. (F) Original frog ERG records (b- and d-waves), obtained during perfusion with Ringer solution in the control period (top traces), 40 μM sulpiride (middle traces) and Ringer solution in the recovery period (bottom traces). Calibration: time – 0.2 s, amplitude – 50 μV.

Effects of 40 μM sulpiride on the intensity-response function of turtle ERG

In the control experiments for this group (n=7) no significant differences were observed between the first and second V – log I function of the b- and d-waves within the same eyecup (two-way repeated measures ANOVA, p>0.05) (Fig. 2A, B). The absolute sensitivity of the responses (determined by their thresholds) as well as their relative sensitivity (determined by I_σ value) were practically identical in both V – log I functions. The same was true for the time characteristics of the responses. This allowed us to evaluate the effect of sulpiride on these parameters using the first V – log I function of the test experiments as a control.

Perfusion with 40 μM sulpiride in the test experiments (n=7) caused no significant changes in the b- and d-wave V – log function compared with the first (control) one (two-way repeated measures ANOVA, p>0.05) (Fig. 2C, D). The absolute and relative sensitivities of the responses were not significantly changed during the blocker application (Table I). These results indicate that 40 μM sulpiride had no significant influence on the V – log I function of the light-adapted turtle ERG, which

contrasted with its suppressive action of the amplitude of the light-adapted frog ERG (Popova 2014b). We did not find any significant effects of 40 μM sulpiride on the time characteristics of the turtle ERG waves as well. Neither the latency nor the implicit time of the ERG b- and d-waves was altered during the perfusion with the blocker (Table II). These negative results may be due to ineffective blockade of dopamine D2-class receptors by 40 μM sulpiride in turtle retina. Thus, we tested the effects of sulpiride at a three times higher concentration (120 μM) on the intensity-response function of the turtle ERG ON and OFF responses.

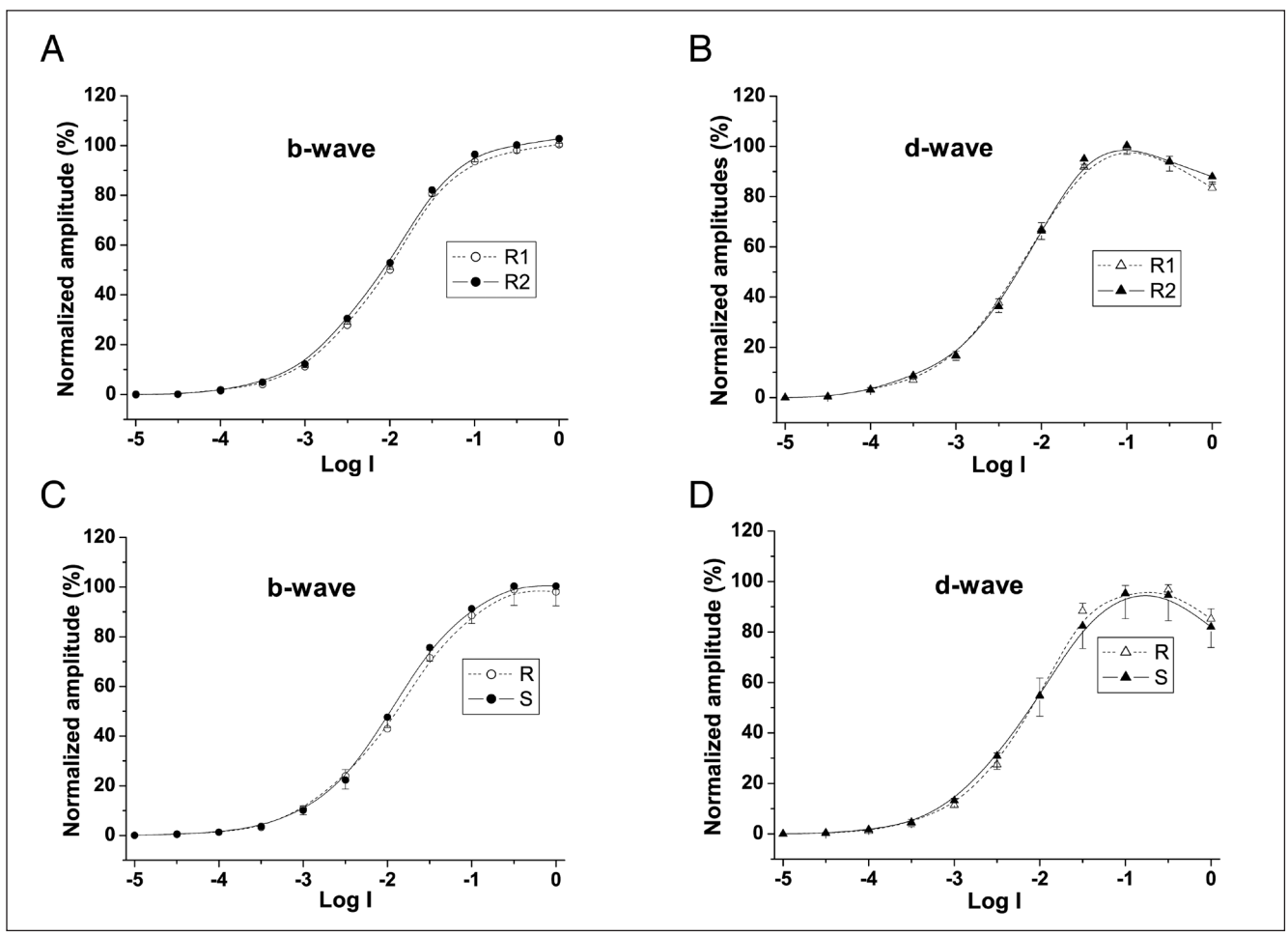

Fig. 2. Effects of 40 μM sulpiride on the V – log I function of the ERG b- and d-waves in light-adapted turtle eyecups. The amplitudes of the ERG waves were normalized to V_{max} of the responses obtained in the control (first) V – log I function in each eyecup. Mean values ± SEM are shown. (A), (B) V – log I function of the b- and d-waves obtained in the control experiments (n=7). The symbols representing the responses obtained during the first (open symbols) and second (filled symbols) V – log I function are denoted in the legends. (C), (D) V – log I function of the b- and d-waves obtained in the test experiments (n=7). The symbols representing the responses obtained during the first (open symbols) and second (filled symbols) V – log I function are denoted in the legends.

Effects of 120 μM sulpiride on the intensity-response function of turtle ERG

The V – log I function obtained during the perfusion with 120 μM sulpiride in test experiments (n=8) showed no significant differences compared to the first V – log I function in the same eyecups (two-way repeated measures ANOVA, $p>0.05$). However, a tendency for enhancement of the b-wave amplitude emerged at higher stimulus intensities (Fig. 3A). All other characteristics of the ERG ON and OFF responses (absolute and relative sensitivities, latency and implicit time) were not significantly changed during sulpiride perfusion (Table I, II). Next, we investigated the effects of a higher concentration (240 μM) of sulpiride in order to determine if the tendency toward b-wave amplitude enhancement was random or a regular phenomenon.

Table I. Changes of the absolute and relative sensitivity of the b- and d-wave V – log I fucntion in turtle and frog experiments with sulpiride application.

ERG wave	Threshold (lgI)		I_σ (lgI)	
	control	sulpiride	control	sulpiride
Turtle 40 μM S (n=7)				
b-wave	-3.37±0.18	-3.35±0.22	-1.91±0.07	-1.98±0.05
d-wave	-3.12±0.25	-3.25±0.21	-2.11±0.08	-2.11±0.11
Turtle 120 μM S (n=8)				
b-wave	-3.29±0.16	-3.23±0.18	-1.96±0.07	-1.97±0.07
d-wave	-3.03±0.21	-3.09±0.19	-2.09±0.07	-2.03±0.09
Turtle 240 μM S (n=12)				
b-wave	-3.59±0.08	-3.66±0.08	-2.03±0.02	-2.08±0.04
d-wave	-3.51±0.07	-3.65±0.12	-2.10±0.02	-2.12±0.02
Frog 240 μM S (n=6)				
b-wave	-4.49±0.17	-4.20±0.14	-3.17±0.28	-3.02±0.29
	p<0.036			
d-wave	-4.36±0.13	-4.00±0.12	-2.54±0.09	-2.54±0.08
	p<0.05			

The threshold and I_σ values of the b- and d-wave V – log I function in control conditions (first V – log I function) are compared to those obtained during the perfusion with sulpiride (S). Results from both turtle and frog experiments are presented. Mean values±SEM are shown. The statistical significance of the differences between control and sulpiride values are evaluated by using paired t-test.

Effects of 240 μM sulpiride on the intensity-response function of turtle ERG

Perfusion with 240 μM sulpiride in test experiments (n=12) caused enhancement of the b- and d-wave amplitude (Fig. 3C, D). The amplitudes of the b-wave were significantly higher than the corresponding values of the first V – log I function (two-way repeated measures ANOVA, p<0.04) with the exception of those obtained at the lowest (threshold) intensities (Table I). The maximal amplitude (V_{max}) of the b-wave was significantly increased (from 158±9.32 to 170±10.78 μV; paired t-test, p<0.042), while the b-wave threshold was not altered (Table I). Thus, it appears that 240 μM sulpiride may modulate the maximal voltage range of the ERG ON response without changing its absolute sensitivity. The amplitude of the d-wave also had higher values during the perfusion with 240 μM sulpiride compared to the corresponding control values, but the difference between them did not reach significance (two-way repeated measures ANOVA, p=0.056). However, the pairwise comparison with Tukey *post hoc* test demonstrated a significant difference between the d-wave amplitude values obtained in the first and second intensity series (p<0.001). The V_{max} value of the second V – log I function (109±10.43 μV) was significantly higher (paired t-test, p<0.045) than that of the first V – log I function (92±3.56 μV), while the threshold values derived from the two curves did not differ significantly (Table I). To further evaluate the effect of sulpiride on the b- and d-wave amplitude and its dependence on stimulus intensity, we compared the relative amplitude change at each stimulus intensity in control and test experiments. The relative amplitude change at each stimulus intensity was estimated by normalization of the values obtained in the second V – log I function to the values obtained in the first (control) function (%) (Fig. 3E, F). There were statistically significant differences between the test and control groups over the entire suprathreshold intensity range (two-way ANOVA, p<0.008 for the b-wave; p<0.003 for the d-wave). This result again confirmed our assertion that 240 μM sulpiride enhanced the amplitude of the b- and d-waves in light-adapted turtle ERG. Two-way ANOVA revealed no significant interaction between stimulus intensity and difference in the relative amplitude change between sulpiride and control experiments. As a consequence, the relative sensitivity of the ERG ON and OFF responses (determined by their I_σ values) was not significantly altered (Table I). Perfusion with 240 μM sulpiride did not change the time characteristics of the ERG responses (Fig. 3G). Neither the latency nor the implicit time of the b- and d-waves was significantly altered during the action of the blocker (Table II). The reversibility of sulpiride effects was investigated in a single experiment, where the perfusion was switched again to Ringer solution. A partial recovery of the b- and d-wave amplitude was observed (Fig. 3G).

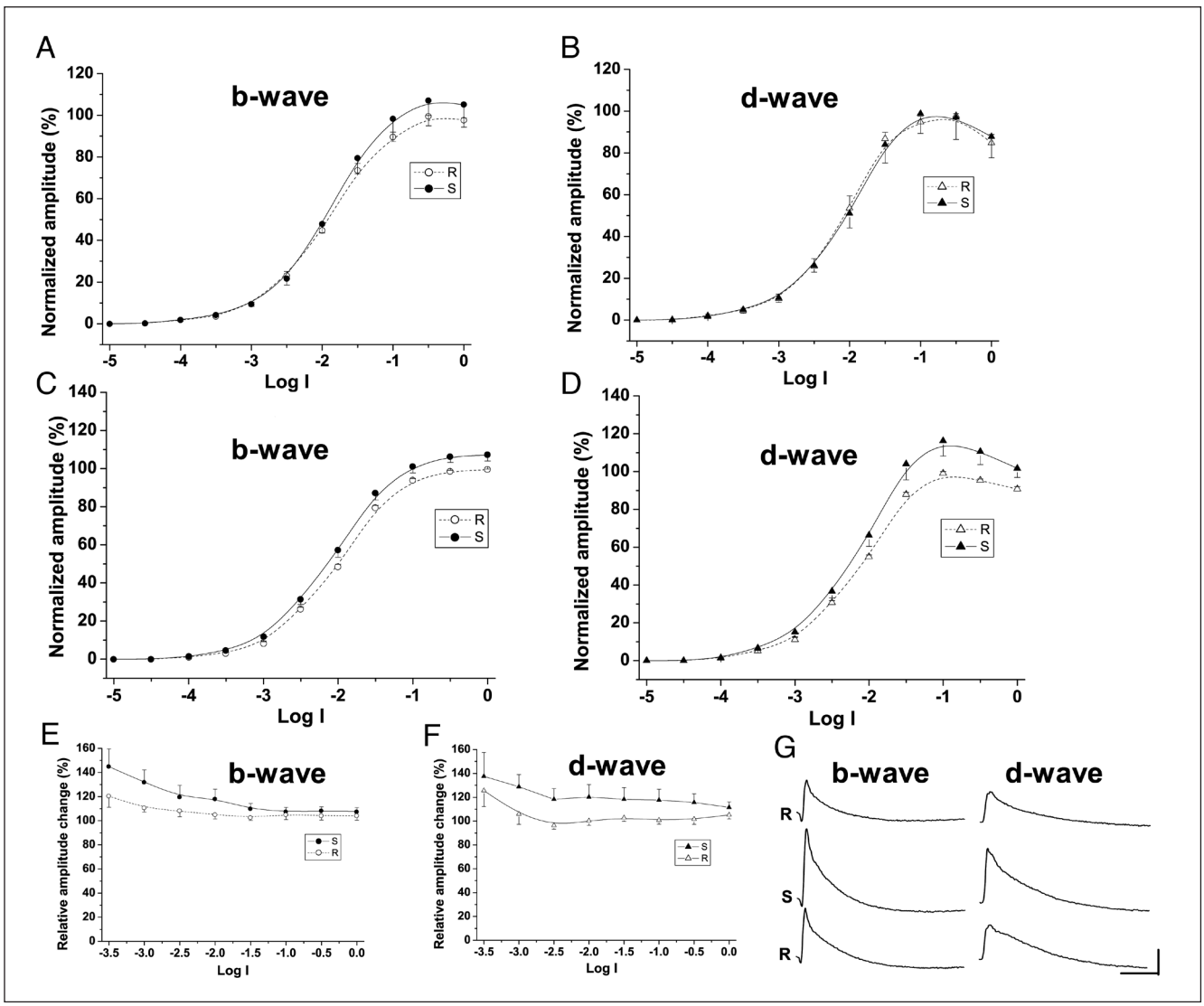

Fig. 3. (A), (B) Effects of 120 μM sulpiride on the V – log I function of the ERG b- and d-waves in light-adapted turtle eyecups. The amplitudes of the ERG waves were normalized to V_{max} of the responses obtained in the control (first) V – log I function in each eyecup. Mean values ± SEM are shown (n=8). The symbols representing the responses obtained during the first (open symbols) and second (filled symbols) V – log I function are denoted in the legends. (C), (D) Effects of 240 μM sulpiride on the V – log I function of the ERG b- and d-waves in light-adapted turtle eyecups. The amplitudes of the ERG waves were normalized to V_{max} of the responses obtained in the control (first) V – log I function in each eyecup. Mean values ± SEM are shown (n=12). All other designations are the same as in (A) and (B). (E), (F) Relative changes of the b- and d-wave amplitude in the control experiments (open symbols) and 240 μM sulpiride experiments (filled symbols). The amplitudes of the ERG waves, obtained at each stimulus intensity during the second V – log I function, were normalized to that obtained during the first V – log I function. Mean values ± SEM are shown. (G) Original ERG records (b- and d-waves), obtained with log I=-2.5 in control conditions (top traces), during perfusion with 240 μM sulpiride (middle traces) and Ringer solution in the recovery period (bottom traces). Calibration: time – 0.25 s, amplitude – 20 μV.

Effects of 240 μM sulpiride on the intensity-response function of frog ERG

As the effect of sulpiride on the turtle ERG wave amplitude was opposite to the effect obtained in the frog ERG, and because it was expressed only at the highest concentration of the blocker, a question arose regarding its specificity. To test this possibility, we studied the effects of 240 μM sulpiride on the intensity-response function of the frog ERG waves and compared them to the effects of 40 μM sulpiride obtained in our previous study (Popova 2014b). In the control experiments for this group (n=6) no significant differences were observed between the first and second V – log I function of the b- and d-waves within the same eyecup (two-way repeated measures ANOVA, $p>0.05$) (Fig. 4A, B). The absolute sensitivity of the responses as well as their relative sensitivity were similar in both V – log I functions. The same was true for the time characteristics of the responses.

Perfusion with 240 μM sulpiride in the test experiments (n=6) caused significant diminution of the amplitude of the b- and d-waves over the whole intensity range (two-way repeated measures ANOVA, $p<0.014$ for b-wave, $p<0.012$ for d-wave) (Fig. 4C, D). The absolute sensitivity of the ERG ON and OFF responses decreased, while their relative sensitivity was not significantly changed (Table I). These results were similar to those we obtained with 40 μM sulpiride in light-adapted frog eyecups (Popova 2014b). However, the depressing effect on the b- and d-wave amplitude was expressed to a greater extent with the higher concentration of the blocker. The relative change of the b- and d-wave amplitude during perfusion with 240 μM sulpiride was significantly greater than that obtained during the perfusion with 40 μM sulpiride (two-way repeated measures ANOVA, $p<0.0001$)

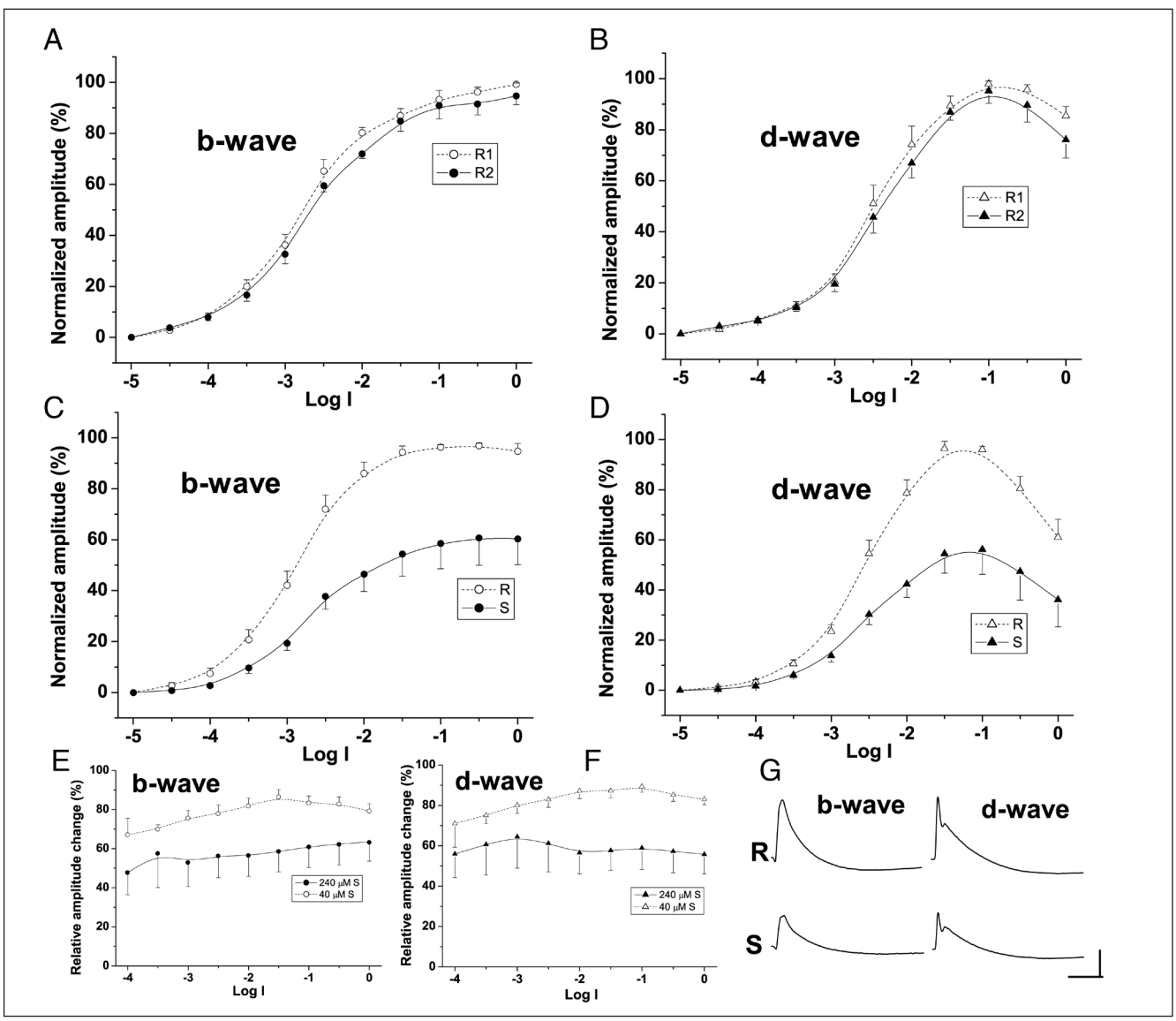

Fig. 4. (A), (B) V – log I function of the ERG b- and d-waves in control experiments of light-adapted frog eyecups. The amplitudes of the ERG waves in the second V – log I function were normalized to V_{max} of the responses obtained in the control (first) V – log I function in each eyecup. Mean values ± SEM are shown (n=6). The symbols representing the responses obtained during the first (open symbols) and second (filled symbols) V – log I function are denoted in the legends. (C), (D) Effects of 240 μM sulpiride on the V – log I function of the ERG b- and d-waves in light-adapted frog eyecups. The amplitudes of the ERG waves were normalized to V_{max} of the responses obtained in the control (first) V – log I function in each eyecup. Mean values ± SEM are shown (n=6). (E), (F) Relative changes of the b- and d-wave amplitude in the 40 μM sulpiride experiments (open symbols) and 240 μM sulpiride experiments (filled symbols). The amplitudes of the ERG waves, obtained at each stimulus intensity during the second V – log I function, were normalized to that obtained during the first V – log I function. The 40 μM sulpiride results are taken from Popova (2014b). Mean values ± SEM are shown. (G) Original ERG records (b- and d-waves), obtained with log I=-2 in control conditions (top traces) and during perfusion with 240 μM sulpiride (bottom traces). Calibration: time – 0.25 s, amplitude – 100 μV.

(Fig. 4E, F). Perfusion with 240 μM sulpiride did not alter the time characteristics of the ERG responses (Fig. 4G). The latency and implicit time of the b- and d-waves showed no significant changes compared to their values during the perfusion with Ringer solution in the control period (Table II). These results are also in agreement with our previously reported results obtained with 40 μM sulpiride in light-adapted frog retina (Popova 2014b).

Table II. Effects of sulpiride on time characteristics of the ERG b- and d-waves in turtle and frog eyes.

ERG wave	Latency (ms)		Implicit time (ms)	
	control	sulpiride	control	sulpiride
Turtle 40 μM S (n=7)				
b-wave	66±8.17	67±4.99	116±11.02	125±12.92
d-wave	46±3.98	51±6.46	105±13.36	106±10.89
Turtle 120 μM S (n=8)				
b-wave	66±7.25	70±6.33	116±9.68	118±11.61
d-wave	49±3.57	50±5.25	92±8.29	94±9.04
Turtle 240 μM S (n=12)				
b-wave	55±0.94	55±0.01	106±2.86	104±3.21
d-wave	30±1.70	31±1.97	120±2.31	114±4.47
Frog 240 μM S (n=6)				
b-wave	72±6.93	75±7.66	162±10.44	172±10.67
d-wave	60±4.51	57±5.94	111±7.33	111±8.91

Latency and implicit time of the ERG waves in control conditions are compared to those obtained during perfusion with sulpiride (S). The log I=-2.5. Results from both turtle and frog experiments are presented. Mean values ± SEM are shown. No significant differences were evaluated between the compared values by using of paired t-test.

DISCUSSION

Our results clearly indicate that there is a marked difference between the dopamine D2-class receptor mediated influences in frog and turtle retina. These differences relate to their sensitivity to antagonists and their functional role in visual information processing, revealed by ERG. Firstly, dopamine D2-class receptors are much more sensitive to sulpiride in frog than turtle retina. We demonstrated that the blocker has marked effects on the frog ERG ON and OFF responses at a concentration of 40 μM, while this concentration and one three times higher (120 μM) is ineffective on the turtle ERG responses. Secondly, the blockade of the D2-class receptors with 240 μM sulpiride has a stimulatory effect on the b-and d-wave amplitude in turtle ERG, while it has a strong depressing effect on the b-and d-wave amplitude in frog ERG.

The described differences between the two species may be due to different expression and distribution patterns of the dopamine D2-class receptors in turtle and frog retina. Wagner et al. (1993) reported that in frog retina the D2-class receptors are well-expressed in photoreceptors and in both plexiform layers, while in turtle retina labeling with D2-antisera is non-specific. Preliminary immunocytochemical data obtained in our laboratory also indicate that the D2, D3 and D4 receptors are predominantly expressed in the outer and inner plexiform layers of frog retina (Zhekova et al. 2015). It is well known that the ON and OFF bipolar cells, whose activities are the primary generator of ERG b- and d-waves, respectively, make their synaptic contacts with the other retinal neurons in both plexiform layers. Thus, modulation of the D2-class receptors in both plexiform layers may easily alter the activity of the ON and OFF bipolar cells in frog retina, which is consistent with our ERG data. Piccolino et al. (1989) reported that in turtle retina, the density of D2-class receptors is about one-fourth of that of D1 receptors and, on the whole, they are a small population of total dopamine receptors. According to our immunocytochemical data in the turtle retina, the distribution of the D2, D3 and D4 receptors is predominantly extrasynaptic neuronal and glial (Zhekova et al. 2015). All these peculiarities in the number and distribution of the D2-class dopamine receptors in turtle retina may account for its lower sen-

sitivity to sulpiride compared to frog retina (revealed by ERG). Some functional studies also lead to the suggestion that the D2-class receptors in turtle retina have lower sensitivity to antagonists than the D2-class receptors in frog retina. Our previous data has shown that 30 μM haloperidol (which preferentially antagonizes D2-class receptor activity) does not significantly change the turtle ERG (obtained with photopic light stimuli), while it has a depressing effect on the frog ERG in the same conditions of light stimulation (Kupenova and Belcheva 1980). Other authors also reported that D2-class receptors in turtle retina have low sensitivity to antagonists. Piccolino et al. (1989) showed that selective D2-class antagonists (metoclopramide, remoxipride or raclopride) in concentrations up to 40 μM usually had no appreciable effect on the light responses of turtle horizontal cells. The lower sensitivity of the D2-class receptors to sulpiride in turtles probably concerns not only the retina but other neuronal structures as well. It has been shown that a concentration of 300 μM sulpiride is needed to block the modulatory effect of dopamine on the olfactory bulb neurons in turtle hemisected brain preparations (Berkowicz and Trombley 2000). A lower permeability of the eyecup preparations to the antagonist may also account for the higher effective concentrations of sulpiride in turtle versus frog eyecups. In our previous study we showed that the enhancing effect of bicuculline ($GABA_A$ receptor antagonist) on the ERG b- and d-wave amplitudes reached maximal expression at a concentration of 300 μM in turtle eyecups versus 50 μM in frog eyecups (Vitanova et al. 2001). This difference in concentration may be due to different sensitivity of the $GABA_A$ receptors and/or different permeability of the eyecups to the blocker.

Our present results clearly show that 240 μM sulpiride has opposite effects on the light-adapted ERG ON and OFF response amplitudes in turtle and frog retina. In our previous works we demonstrated that selective D2-class receptor blockade with 40 μM sulpiride has a suppressing effect on the cone-dominated b- and d-wave amplitudes in both dark and light-adapted frog ERG (Popova and Kupenova 2013, Popova 2014b). Our present results with 240 μM sulpiride confirm these data and show that the depressing effect is stronger when the blocker concentration is higher. Thus, it appears that the endogenous dopamine acting through D2-class receptors has a dose-dependent enhancing effect on the cone-dominated ON and OFF responses in frog ERG. This effect is expressed in the increased absolute sensitivity and maximal voltage range of the responses with no change in their relative sensitivity and time characteristics. With its stimulatory action on the cone-mediated ERG responses in frog retina, dopamine can serve as a chemical messenger for light adaptation as has been proposed for many other species (for review: Popova 2014a, Witkovsky 2004). In turtles, dopamine action mediated by D2-class receptors is different. In the present work, we showed that the blockade of D2-class receptors with the highest concentration of sulpiride (240 μM) led to an enhancement of the b- and d-wave amplitude over the whole intensity range except for the lowest (threshold) intensities. This means that the endogenous dopamine acting through D2-class receptors has a depressing effect on the cone-mediated ON and OFF responses in turtle ERG. This effect is expressed in compressing the maximal voltage range of the responses without altering their absolute and relative sensitivity as well as their time characteristics. Our results obtained in turtle ERG are in line with the results of Kim and Jung (2012) in goldfish ERG, where 200 μM sulpiride caused an increase of the light-adapted b-wave amplitude of approximately 44%. An increase of the dc-component of the ERG ON response under the influence of 10 μM sulpiride has also been seen in light-adapted goldfish by Mora-Ferrer and Behrend (2004). The authors suggest that the effect on the dc-component is due to a decrease of the OFF bipolar cell response caused by sulpiride, because the blocker dampened the ERG OFF response (push-pull model of Sieving et al.1994). This model could not explain our results, because 240 μM sulpiride had an enhancing effect on the amplitude of both the ERG ON and OFF responses. The proposed inhibitory effect of the endogenous dopamine on the ERG b- and d-wave generating mechanisms in turtle and fish retina may be due to activation of the D2 autoreceptors expressed by dopaminergic cells, which function to inhibit dopamine release (Derouiche and Asan 1999, Veruki 1997, Wang et al. 1997). Diminished dopamine release could prevent the excitatory action of dopamine on the b-wave generating mechanisms that is mediated by the D1-class receptors in light-adapted fish retina (Kim and Jung 2012). Unfortunately, we could not find any data concerning the effects of the D1-class receptor manipulation on turtle ERG. If the activation of the D1-class receptors has similar excitatory action on the b- and d-wave generating mechanisms in turtle retina, this could account for the effect of sulpiride obtained in the present study. Further studies are needed to fully clarify the precise mechanism underlying the dopamine action mediated by D2-class receptors in turtle retina.

CONCLUSIONS

Our results clearly showed that there is a marked difference between D2-class receptor-mediated pathways in turtle and frog retina. This difference relates to their sensitivity to antagonists and the consequences of their blockade on global retinal function (revealed

by ERG). While D2-class receptor blockade with a high dosage of sulpiride (240 μM) enhanced the amplitude of ERG ON and OFF responses in turtle ERG, a marked diminution of ON and OFF response amplitudes was seen in frog ERG, even with a small dosage of the blocker (40 μm).

REFERENCES

Barton AC, Black LE, Sibley DR (1991) Agonist-induced desensitization of D2 dopamine receptors in human Y-79 retinoblastoma cells. Mol Pharmacol 39: 650–658.

Berkowicz DA, Trombley PQ (2000) Dopaminergic modulation at the olfactory nerve synapse. Brain Res 855: 90–99.

Derouiche A, Asan E (1999) The dopamine D2 receptor subfamily in rat retina: ultrastructural immunogold and in situ hybridization studies. Eur J Neurosci 11: 1391–1402.

Frishman LJ (2006) Origins of the electroretinogram. In: Heckenlively JR, Arden GB, editors. Principles and Practice of Clinical Electrophysiology of Vision. 2nd ed. London: MIT Press; p.139–183.

Frishman LJ (2013) Electrogenesis of the electroretinogram. In: Ryan SJ, Hinton DR, Sadda SR, Schachat AP, Wilkinson CP, Wiedemann P, editors. Retina. 5th ed. Elsevier Health Sciences; p. 177–201.

Gardner B, Liu ZF, Jiang D, Sibley DR (2001) The role of phosphorylation/dephosphorylation in agonist-induced desensitization of D1 dopamine receptor function: evidence for a novel pathway for receptor dephosphorylation. Mol Pharmacol 59: 310–321.

Jackson CR, Ruan GX, Aseem F, Abey J, Gamble K, Stanwood G, Palmiter RD, Iuvone PM, McMahon DG (2012) Retinal dopamine mediates multiple dimensions of light-adapted vision. J Neurosci 32: 9359–9368.

Kim DY, Jung CS (2012) Gap junction contributions to the goldfish electroretinogram at the photopic illumination level. Korean J Physiol Pharmacol 16: 219–224.

Kolb H, Netzer E, Ammermüller J (1997). Neural circuitry and light responses of the dopamine amacrine cell of the turtle retina. Mol Vis 3: 6.

Kupenova P, Belcheva S (1980) Effects of methylergometrine (ME), haloperidol (HA) and phentolamine (PH) on the frog's and turtle's ERG. Proceedings of the International Union of Physiological Sciences, vol 14, p 533, No 2146, XXVIII International Congress, Budapest.

Kupenova P, Vitanova L, Popova E (2010) GABAa and GABAc receptor-mediated modulation of responses to color stimuli: electroretinographic study in the turtle *Emys orbicularis*. J Neural Trans 117: 431–444.

Kupenova TN (2011) An inductive algorithm for smooth approximation of functions. Commun JINR, Dubna, E11-2011-97.

Lavoie J, Illiano P, Sotnikova TD, Gainetdinov RR, Beaulieu JM, Hébert M (2014) The electroretinogram as a biomarker of central dopamine and serotonin: potential relevance to psychiatric disorders. Biol Psychiatry 75: 479–486.

Mora-Ferrer C, Behrend K (2004) Dopaminergic modulation of photopic temporal transfer properties in goldfish retina investigated with the ERG. Vision Res 44: 2067–2081.

Naka KI, Rushton WAH (1966) S-potentials from colour units in the retina of fish (Cyprinidae). J Physiol 185: 536–555.

Perry B, George JS (2007) Dopaminergic modulation and rod contribution in the generation of oscillatory potentials in the tiger salamander retina. Vision Res 47: 309–314.

Piccolino M, Demontis G, Witkovsky P, Strettoi E, Cappagli GC, Porceddu ML, De Montis MG, Pepitoni S, Biggio G, Meller E, Bohmaker K (1989) Involvement of D1 and D2 dopamine receptors in the control of horizontal cell electrical coupling in the turtle retina. Eur J Neurosci 1: 247–257.

Popova E (2014a) Role of dopamine in distal retina. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 200: 333–358.

Popova E (2014b) Effects of dopamine receptor blockade on the intensity-response function of electroretinographic b- and d-waves in light adapted eyes. J Neural Trans 121: 233–244.

Popova E, Kupenova P (2013) Effects of dopamine receptor blockade on the intensity-response function of ERG b- and d-waves in dark adapted eyes. Vision Res 88: 22–29.

Sieving PA, Murayama K, Naarendorp F (1994) Push-pull model of the primate photopic electroretinogram: A role for hyperpolarizing neurons in shaping the b-wave. Vis Neurosci 11: 519–532.

Veruki M (1997) Dopaminergic neurons in the rat retina express dopamine D2/3 receptors. Eur J Neurosci 9: 1096–1100.

Vitanova L, Kupenova P, Haverkamp S, Popova E, Mitova L, Wassle H (2001) Immunocytochemical and electrophysiological characterization of GABA receptors in the frog and turtle retina. Vision Res 41: 691–704.

Wagner HJ, Luo BG, Ariano MA, Sibley DR, Stell WK (1993) Localization of D2 dopamine receptors in vertebrate retinae with anti-peptide antibodies. J Comp Neurol 331: 469–481.

Wang Y, Harsanyi K, Mangel SC (1997) Endogenous activation of dopamine D_2 receptors regulates dopamine release in the fish retina. J Neurophysiol 78: 439–449.

Witkovsky P (2004) Dopamine and retinal function. Doc Ophthalmol 108: 17–40.

Zhekova D, Popova E, Kupenova P, Vitanova L (2015) Comparative investigation of D2 dopamine receptors in frog and turtle retina: immunocytochemical and electrophysiological study. Folia Medica 57, suppl 3, 14.