Effects of dopamine D₁ receptor blockade on the intensity-response function of ERG b- and d-waves under different conditions of light adaptation

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ABSTRACT

The effect of dopamine D₁ receptor blockade by SCH 23390 on the V–log I function of the ERG b- and d-waves was investigated in dark and light adapted frog eyes. We obtained that the blocker enhanced the amplitude of the b- and d-waves in both conditions of adaptation. The enhancing effect of the blocker was more pronounced on the rod-than cone-dominated responses for the both ERG waves. The absolute sensitivity of the b-wave was not altered, but that of the d-wave was significantly increased. The intensity-response function of the b-wave, but not that of the d-wave, was shifted to the left along the intensity axis. The b-wave V–log I function had steeper slope and narrower dynamic range in both dark and light adapted eyes after the D₁ receptor blockade. The results obtained indicate that the endogenous dopamine, acting through D₁ receptors, does not play a crucial role in the process of retinal adaptation, although it changes in a specific manner the intensity-response function of both the ERG b- and d-waves.

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1. Introduction

Dopamine is the predominant catecholamine in the vertebrate retina. It is released by a unique set of dopaminergic amacrine/interplexiform neurons (for review: Witkovsky, 2004). Dopamine acts through five subtypes of dopamine receptors designated as D₁–D₅. These receptors are grouped into two subfamilies: (1) the D₁–D₃ or D₂-like subfamily receptors. The rod and cone photoreceptors have D₁ subfamily receptors, while bipolar, horizontal, amacrine and ganglion cells have D₁ receptors (for review: Witkovsky, 2004). Many data indicate that the endogenous dopamine release is low in darkness and increases during retinal exposure to constant or flickering light (Boatright, Hoel, & Iuvone, 1989; Boelen, Boelen, & Marshall, 1998; Gibson, 1990; Godfrey & Wurtman, 1988; Kirsh & Wagner, 1989; Kolbinger & Weiler, 1993; Kramer, 1971; Mills et al., 2007; Parkinson & Rando, 1983; Puppala, Maaswinkel, Mason, Legan, & Li, 2004; Witkovsky, Nicholson, Rice, Bohmaker, & Meller, 1993). These observations suggest that dopamine may be related to the process of retinal adaptation. Some data indicate that application of exogenous dopamine changes the activity of single retinal neurons in a manner similar to that during light adaptation (Dong & McReynolds, 1991; Hamson, Vaney, & Weiler, 1992; He, Weiler, & Vaney, 2000; Hu, Pan, Volgyi, & Bloomfield, 2010; Lasater & Dowling, 1985; Negishi, Teranishi, & Kato, 1983; Piccolino, Neyton, & Gerschenfeld, 1984; Vaquero, Pignatelli, Partida, & Ishida, 2001; Witkovsky, Stone, & Besharse, 1988). However, other data indicate that it induces changes as does a prolonged period of complete darkness (Mangel & Dowling, 1985; Mangel & Dowling, 1987; Yang, Tornqvist, & Dowling, 1988).

It is well known that vertebrate retinal sensitivity can be quantitatively analyzed by recording of electroretinogram (ERG). ERG consists of many components, but two of them are most prominent in response to long lasting stimuli: a b-wave (in response to stimulus onset) and a d-wave (in response to stimulus offset). These components are usually used for assessment of the retinal ON and OFF channel activity. The dopamine participation in the process of retinal adaptation can be readily evaluated by application of dopaminergic drugs and following up the changed of the ERG b- and d-waves, obtained under different conditions of light adaptation. More reliable information can be obtained by blocking the endogenous dopaminergic transmission than by application of exogenous dopamine or its agonists, because dopamine receptors undergo desensitization as a result of prolonged exposure to agonist (Ko, Seeman, Sun, & Kapur, 2002). Most of the authors reported that depletion of retinal dopamine (with 6-OHDA treatment) or application of the nonselective dopamine antagonist haloperidol had no effect on the threshold dark adapted ERG (goldfish: Lin & Yazulla, 1994; Yazulla, Lin, & Studholme, 1996; zebrafish: Li & Dowling, 2000; cat: Naarendorp, Hitchcock, & Sieving, 1993), although the behaviorally measured visual sensitivity was decreased (Li & Dowling, 2000). There is no agreement about the...
effects of the dopaminergic retinal blockade on the suprathreshold ERG, obtained under different conditions of adaptation. Some authors failed to observe any effect on the b-wave amplitude in both dark and light adapted eyes (Li & Dowling, 2000; Lin & Yazulla, 1994; Yazulla et al., 1996), although an increase of the perceived brightness was demonstrated in light-adapted conditions (Lin & Yazulla, 1994). Other authors obtained an increase of the b-wave amplitude (monkey: Bodis-Wollner, Marx, & Ghilardi, 1989; cat: Naarendorp et al., 1993; Schneider & Zrenner, 1991; Skrandies & Wässle, 1988; rabbit: Nakagawa et al., 1994; Oliver, Jolicoeur, Lafond, Drumheller, & Brunette, 1987) and a left shift of its V−log I curve, indicating increased relative sensitivity of the response (Schneider & Zrenner, 1991). Still other authors reported a decrease of the b-wave amplitude (frog: Citron, Erinoff, Rickman, & Brecha, 1985; Kupenova & Belcheva, 1981; mudpuppy: Wachmeister, 1981; mice: Adachi-Usami, Ikeda, & Satoh, 1990; Mizota & Adachi-Usami, 1993) with the effect particularly marked in the range of middle and higher stimulus intensities (chicken: Wioland, Rudolf, & Bonaventure, 1990). The discrepancy in the results cited might be due to species differences including involvement of different kinds of dopamine receptors.

There are no comparative studies concerning the effects of selective D1 and D2 dopamine receptor antagonists on the ERG b- and d-waves under different conditions of light adaptation. A few studies were performed under one and the same level of background illumination, and the results obtained are contradictory. Some authors obtained that the selective D1 dopamine antagonist SCH 23390 diminished the amplitude of the ERG b-wave (rabbit: Huppe-Gourgues et al., 2005) or to increase the amplitude of the b-wave with shifting of its V−log I function to the left along the intensity axis (Schneider & Zrenner, 1991). Changes of the d-wave amplitude were not followed up in any of these studies.

In the present study we investigated the effects of the selective D1 receptor antagonist SCH 23390 on the intensity-response function of the frog ERG b- and d-waves in conditions of dark and light adaptation.

2. Material and methods

The experiments were carried out on 62 eyecup preparations of frog (Rana ridibunda), continuously superfused with Ringer solution at a rate of 1.8−2.0 ml/min and supplied with moistened O2 (for details see Popova & Kupenova, 2009). The D1 dopamine receptors were blocked using the D1 dopamine antagonist SCH 23390 (Sigma), dissolved in Ringer solution to a concentration of 10 µM. This concentration was the lowest tested one that had a significant effect on the ERG waves. The same concentration was used by other authors working on amphibian retina (Krizaj & Witkovsky, 1993; Perry & George, 2007).

2.1. Light stimulation

Diffuse white light stimuli (150 W tungsten halogen lamp) with 5 s duration were presented repeatedly at interstimulus interval of 25 s. The test stimulus intensity (I) was changed in an ascending manner over a range of 11 log units by means of neutral density filters. The maximal intensity (denoted by 0) was 6 × 10^8 quanta s^{-1} μm^{-2} at the plane of the retina. The test stimuli

Fig. 1. Spectral sensitivity curves of the b- and d-waves in dark and light adapted eyes. The spectral sensitivity was determined as reciprocal value of the threshold. The threshold was obtained using 5 μV (filled squares) and 10 μV (filled circles) criterion amplitude. The threshold for the second, higher intensity, part of the dark adapted V−log I function was determined using 75 μV criterion amplitude for the b-wave and 25 μV for the d-wave (open triangles). These criterion amplitudes were chosen, because they were reached immediately after the beginning of the second limb of the curves.

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were presented in the dark or under diffuse white background illumination with intensity of $2.4 \times 10^6$ quanta s$^{-1}$ µm$^{-2}$, which was sufficient to saturate the rods (Fain, 1976; Hood & Hock, 1975). These light stimulation conditions allowed us to obtain rod-dominated responses (using low $I_t$ in dark adapted eyes) and cone-dominated responses (using high $I_t$ in dark adapted eyes or using rod-saturating background). The type of photoreceptor input was proved by ERG response spectral sensitivity assessment. A clear Purkinje shift (from 500 nm to 568 nm) was demonstrated during transition from dark to light adaptation. The same was true when low intensity stimuli were substituted by high intensity ones in the dark adapted eyes (Fig. 1).

### 2.2. Experimental procedure

The frogs were dark adapted for 24 h and then the eyecup preparation was made under dim red light. The test light stimulation started after a period of new adaptation – 30 min in the dark or 15 min under photopic background.

In order to test the dynamics of the D1 blocker effect, in a group of experiments the effects of 10 µM SCH 23390 (Ringer solution in the controls resp.) were followed for a period of 26 min in conditions of dark adaptation using a constant test stimulus ($I_t = 6.0$).

In the other groups of experiments after the period of dark or light adaptation, $V$–$\log I$ function of the ERG waves was obtained using stimuli with increasing intensity (first series). The procedure of adaptation and test stimulation was then repeated and second $V$–$\log I$ function was obtained (second series). In the control experiments both series were obtained during perfusion with Ringer solution. In the test experiments the first $V$–$\log I$ function was obtained during Ringer solution perfusion and the second one – during perfusion with 10 µM SCH 23390. The perfusion was switched from Ringer solution to SCH 23390 12 min before the beginning of the second intensity series, when the effect of the blocker was fully developed (see Fig. 2).

### 2.3. ERG recording and data analysis

The electroretinograms were recorded by means of non polarized Ag|AgCl electrodes at bandpass of 0.1–1000 Hz and digitized at 1 kHz. The amplitude of the ERG waves was measured from peak to peak. For assessment of the relative amplitude change at each $I_t$ the values obtained in the second intensity series were normalized to the values obtained in the first series (%). This was done for both the control and test experiments. The peak amplitudes of the responses to stimuli of different $I_t$ were used for $V$–$\log I$ function evaluation. The absolute sensitivity of the ERG responses was assessed by their thresholds, estimated using two criterion response amplitudes; 5 µV and 10 µV. As the amplitude of the rod dominated d-wave was very low and did not reach 10 µV in all of the experiments, this criterion amplitude was not used for absolute sensitivity assessment of the scotopic d-wave. The b-wave $V$–$\log I$ function was fitted to the Naka–Rushton equation: $V = V_{\text{max}} \cdot \frac{I_t}{I_t + I_n}$, where $V$, amplitude of the ERG waves; $V_{\text{max}}$, its maximum; $I_t$, stimulus intensity above the background; $I_n$, stimulus intensity required to produce half-maximum amplitude; $n$, exponent, related to the steepness of the $V$–$\log I$ function (Naka & Rushton, 1966). The value of $I_n$ was used as an index of the response relative sensitivity. The dynamic range of the responses was estimated as intensity span of the responses with 5–95% $V_{\text{max}}$ amplitude. The $V$–$\log I$ function of the d-wave had a more complex character and it could not be fitted well to Naka–Rushton equation (for details...
see Popova, Kupenova, Vitanova, & Mitova, 1995). Individual V–log I curves of the d-wave were constructed, then \( V_{\text{max}} \) and \( I_r \), producing 0.5 \( V_{\text{max}} \) (\( I_r \)), were obtained after curve smoothing using B-spline. In the dark adapted eyes, where the V–log I curve had two limbs (rod- and cone-dominated), the whole curve \( I_r \) point was always on the second limb of the curve. The complex character of the d-wave V–log I function did not allow us to determine its dynamic range.

For statistical evaluation of the data, Student's t-test, One- and Two-Way ANOVA with Bonferroni test (alpha = 0.05) were used.

### 3. Results

#### 3.1. Dark adaptation group

#### 3.1.1. Dynamics of the SCH 23390 effects

This group of experiments were carried out in order to evaluate the time course of the blocker effects. ERG was firstly recorded for 9 min in control conditions (during perfusion with Ringer solution) and then during perfusion with solution of 10 \( \mu \)M SCH 23390 for another 26 min. Switching the perfusion to 10 \( \mu \)M SCH 23390

![Figure 3](image)

**Fig. 3.** Effects of SCH 23390 on the V–log I function of the b- and d-wave in the ERG, obtained in dark adapted eyes. Results of both control experiments (a and b; \( n = 10 \)) and test experiments (c and d; \( n = 12 \)) are represented. The amplitudes of the ERG waves are normalized to \( V_{\text{max}} \) of the responses obtained during the first series of the experiments. Mean values ± SEM are shown. The symbols, representing the responses obtained during the first and second intensity series, are denoted in the legends.

<table>
<thead>
<tr>
<th>Adaptation</th>
<th>ERG wave</th>
<th>Threshold (log ( I_t ))</th>
<th>( I_r ) (log ( I_t ))</th>
<th>( n )</th>
<th>Dynamic range (log units)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ringer</td>
<td>SCH 23390</td>
<td>Ringer</td>
<td>SCH 23390</td>
<td>Ringer</td>
</tr>
<tr>
<td>DA</td>
<td>b-wave</td>
<td>(-11.10 \pm 0.08^*)</td>
<td>(-11.02 \pm 0.05^*)</td>
<td>(-7.66 \pm 0.16)</td>
<td>(-8.28 \pm 0.15)</td>
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<tr>
<td></td>
<td></td>
<td>(-10.83 \pm 0.09)</td>
<td>(-10.74 \pm 0.04)</td>
<td>( p &lt; 0.000005)</td>
<td>( p &lt; 0.05)</td>
</tr>
<tr>
<td></td>
<td>d-wave</td>
<td>(-9.99 \pm 0.27)</td>
<td>(-10.57 \pm 0.21)</td>
<td>(-5.61 \pm 0.07)</td>
<td>(-5.72 \pm 0.04)</td>
</tr>
<tr>
<td>LA</td>
<td>b-wave</td>
<td>(-4.77 \pm 0.07^*)</td>
<td>(-4.73 \pm 0.06^*)</td>
<td>(-3.24 \pm 0.08)</td>
<td>(-3.48 \pm 0.07)</td>
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<tr>
<td></td>
<td></td>
<td>(-4.51 \pm 0.06)</td>
<td>(-4.51 \pm 0.06)</td>
<td>( p &lt; 0.0003)</td>
<td>( p &lt; 0.002)</td>
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<tr>
<td></td>
<td>d-wave</td>
<td>(-4.72 \pm 0.08)</td>
<td>(-4.77 \pm 0.09)</td>
<td>(-2.83 \pm 0.11)</td>
<td>(-2.96 \pm 0.10)</td>
</tr>
</tbody>
</table>

*5 \( \mu \)V threshold criterion amplitude; other threshold values – 10 \( \mu \)V criterion amplitude. The statistic significance of the differences between the values before and after SCH 23390 treatment is evaluated.
caused marked increase of the b- and d-wave amplitude, which reached a plateau at the 12th minute from the beginning of the blocker application (Fig. 2). The SCH 23390 effects on the ERG waves were relatively stable until the end of the perfusion period. The b- and d-wave amplitudes recovered to a great degree during reperfusion with Ringer solution (Fig. 2, inset).

3.1.2. Effects of SCH 23390 on the intensity-response function

In the control experiments of this group the V–log I function of the b- and d-waves showed no significant differences between the first and second intensity series in one and the same eyecup with the exception of a slight enhancement of the d-wave amplitude in the lower, rod-dominated part of the curve during the second series (Fig. 3a and b). The absolute sensitivity of the responses (determined by their thresholds) as well as their relative sensitivity (determined by IΔr value) were practically identical in both intensity series. The same was true for the dynamic range of the b-wave and the time course of the responses. This allowed us to evaluate the effect of dopamine antagonist on these parameters using the first series of the test experiments as a control one.

The perfusion with 10 μM SCH 23390 in the test experiments caused significant increase of the b- and d-wave amplitude at all stimulus intensities (Two-way ANOVA p < 0.000001) except for the lowest ones for the b-wave (I<sub>t</sub> = −11.5, −11, −10.5) and the middle ones for the d-wave (I<sub>t</sub> = −8, −7.5, −7) (Fig. 3c and d). The absolute sensitivity of the b-wave was not significantly altered, which is evident from the unaltered thresholds values (Table 1). The SCH 23390 enhancing effect on the b-wave amplitude started at stimulus intensities, which were ~1 log unit above the threshold. On the other hand, the absolute sensitivity of the d-wave was increased (Table 1). The stimulating effect of SCH 23390 on the b- and d-wave amplitude showed clear intensity dependence. It was greatest in the lower part of the intensity range, where the responses were mediated by rods and was less pronounced at higher intensities, where the responses were mediated by cones (Fig. 4a and b). The Two-Way ANOVA showed significant interaction between the effect of the blocker and stimulus intensity (p < 0.000001) and the One-Way ANOVA with Bonferroni test revealed statistically significant difference (p < 0.05) between the relative amplitude change at lower (I<sub>t</sub> = −10, −9.5, −9) and higher (I<sub>t</sub> above −6.0) intensities.

Perfusion with SCH 23390 increased the rod-mediated d-wave amplitude to a greater extent than the rod-mediated b-wave. This resulted in significantly lowered b/d amplitude ratio (Two-way ANOVA p < 0.01) in the rod-dominated part of the intensity curve (Fig. 4c). An interesting effect was observed in the intensity range (I<sub>t</sub> = −8; I<sub>t</sub> = −7.5; I<sub>t</sub> = −7), where transition from rod to cone dominated responses occurred. In this range the b-wave amplitude was enhanced to the same (maximal) degree as that obtained at lower intensities. On the other hand, the d-wave amplitude was not significantly altered. As a consequence the b/d amplitude ratio was significantly increased (p < 0.03) in this intensity range (Fig. 4c). In the cone dominated part of the V–log I function, the D<sub>1</sub> blocker enhanced the b- and d-wave amplitudes to the same extent. Thus the b/d amplitude ratio remained unchanged in the range of higher stimulus intensities.

The V–log I curve of the b-wave had steeper slope and narrowed dynamic range during the D<sub>1</sub> receptor blockade (Table 1). This means that the maximal contrast sensitivity (near I<sub>0</sub> point) was in-
increased, but the response amplitude was saturated at lower stimulus intensities. The curve was shifted to the left along the intensity axis, indicating increased relative sensitivity of the ON response (Fig. 4d). This effect was demonstrated by the significantly lower value of $I_r$ (Table 1). However, the relative sensitivity of the d-wave remained unchanged. The latter was evident from the unaltered position of the d-wave $V$–log $I$ curve along the intensity axis (Fig. 4e) and unchanged value of $I_r$ (Table 1).

The perfusion with SCH 23390 had different effects on the time course of the b-wave depending on the photoreceptor input. It slowed the time course of the rod-, but not cone dominated b-wave without altering its latency (Fig. 4f). The implicit time of the b-wave was significantly increased at lower intensities (at $I_t = -9.5$ from $777 \pm 40.16$ to $883 \pm 35.57$ ms, $p < 0.025$), but no significant difference was observed at higher intensities (at $I_t = -4$ it was $296 \pm 13.36$ and became $283 \pm 21.08$ ms). Thus, the initial difference between the implicit times of the rod- and cone-dominated b-wave was augmented after the D1 receptor blockade. The effect of SCH 23390 on the time course of the d-wave did not show clear dependence on photoreceptor input. The latency of the response was not changed, but its implicit time was significantly increased both at higher intensities (at $I_t = -4$ from $213 \pm 16.69$ to $235 \pm 19.92$ ms, $p < 0.002$) and lower intensities (at $I_t = -9.5$ from $850 \pm 34.08$ to $1020 \pm 58.94$ ms, $p < 0.005$). Because the lengthening of the implicit time of the rod-dominated d-wave was expressed to a greater degree as compared to the cone-dominated one ($p < 0.01$), the difference between the two implicit times was augmented.

### 3.2. Light adaptation group

In the control experiments of this group the $V$–log $I$ function of the b- and d-waves showed no significant differences between the first and second intensity series in one and the same eyecup (Fig. 5a and b). The absolute and relative sensitivity of the b- and d-waves as well as the dynamic range of the b-wave were not significantly different in the two intensity series.

The perfusion with 10 µM SCH 23390 in the test experiments caused significant increase of the b- and d-wave amplitude at all stimulus intensities (Two-way ANOVA $p < 0.000001$) except for the lowest two ($I_t = -5$ and $I_t = -4.5$) (Fig. 5c and d). The stimulating effect of the blocker was expressed to the same extent as that obtained in the dark adapted eyes, when high intensity stimuli were applied and the responses were cone-dominated (compare Fig. 4a and b and Fig. 5a and b). The b-wave threshold was not altered (Table 1) indicating that the D1 receptor blockade had no significant effect on the absolute sensitivity of the photopic ON response in frog ERG. The d-wave threshold was also not significantly changed, when lower criterion amplitude (5 µV) was used for its assessment, but it was significantly lowered, when higher criterion amplitude (10 µV) was chosen (Table 1). This suggests that the action of endogenous dopamine upon the photopic ERG OFF response is developed at lower response amplitude as compared to the ON response.

The stimulating effect of SCH 23390 on the b-wave amplitude was not equally expressed over the whole intensity range (Fig. 6a). The Two-Way ANOVA showed significant interaction be-

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**Fig. 5.** Effects of SCH 23390 on the $V$–log $I$ function of the b- and d-wave in the ERG, obtained in light adapted eyes. Results of both control experiments (a and b; n = 10) and test experiments (c and d; n = 9) are represented. The amplitudes of ERG waves are normalized to $V_{\text{max}}$ of the responses in the first series of the experiments. Mean values ± SEM are shown. The symbols, representing the responses obtained during the first and second intensity series, are denoted in the legends.
between the effect of the blocker and stimulus intensity ($p < 0.008$). The effect was maximal within the intensity range close to the $I_r$ point ($I_r = -3.5$ and $I_r = -3$). The relative increase of the b-wave amplitude in this intensity range differed significantly ($p < 0.05$) from that obtained at higher intensities (with the exception of $I_r = -2.5$). As a consequence the b-wave $V$-$\log I$ curve was shifted to the left along the intensity axis (Fig. 6d) and the $I_r$ value was significantly decreased (Table 1). This indicated that the relative sensitivity of the response was increased during the D1 receptor blockade. The b-wave $V$-$\log I$ curve had much stepper slope (Fig. 6d) and narrowed dynamic range (Table 1), which resembled the effect obtained in dark adapted eyes.

The stimulating effect of SCH 23390 on the d-wave amplitude did not show clear intensity dependence (Fig. 6b). The Two-Way ANOVA did not show significant interaction between the effect of the blocker and stimulus intensity. The position of the d-wave $V$-$\log I$ curve along the intensity axis was practically the same (Fig. 6e) and the value of $I_r$ did not change significantly (Table 1). This indicated that D1 receptor blockade did not alter significantly the relative sensitivity of the photopic OFF response, which was similar to the effect, obtained in the dark adapted eyes. The later result was expected, because the $I_r$ point in the dark adapted eyes was always on the second, cone-mediated part of the d-wave $V$-$\log I$ curve. The enhancing effect of SCH 23390 on the d-wave amplitude was expressed to the same extent as that on the b-wave, leading to unchanged b/d amplitude ratio over the whole intensity range (Fig. 6c).

Perfusion with SCH 23390 changed the time course of the ERG waves in a way identical to that obtained for cone-dominated responses of the dark adapted eyes. Neither latency nor the implicit time of the b-wave were changed significantly (Fig. 6f). The implicit time of the d-wave was slightly delayed (at $I_r = -3$ from $144 \pm 6.93$ to $169 \pm 7.11$ ms, $p < 0.05$), while its latency was not altered significantly.

4. Discussion

Our results clearly demonstrate that the blockade of dopamine D1 receptors by SCH 23390 enhances the amplitude of the ERG b- and d-waves in both dark and light adapted frog retina. This indicates that endogenous dopamine, acting through D1 receptors, has inhibitory action on the mechanisms, responsible for generation of these ERG waves regardless of the state of retinal adaptation and the type of the photoreceptor input. However, it has been shown by other authors that depletion of retinal dopamine or application of the nonselective dopamine antagonist haloperidol decreases the b- and d-wave amplitude in frog retina (Citron et al., 1985; Kupenova & Belcheva, 1981), suggesting that the endogenous dopamine has an overall enhancing effect on the ERG waves. We might speculate that the later effect is probably mediated through the other types of dopamine receptors (excluding the D1 receptors). Our results are consistent with the results obtained in monkey retina, where haloperidol increased the amplitude of the flash ERG b-wave in both dark and light adapted eyes (Bodis-Wollner et al., 1989). Since the authors used only one stimulus intensity, they were not able to examine separately the rod and cone-mediated responses. Our data are at odds with the results obtained in amphibian retina, where SCH 23390 did not change significantly the photopic ERG ON and OFF response (Perry & George, 2007). They differ also from the results obtained in rabbit retina, where SCH 23390 reduced the b-wave amplitude in scotopic (Marmor et al., 1988) or mesopic (Huppe-Gourgues et al., 2005) conditions. But the latter authors have also found that the selective D2 receptor agonists (SKF38393 and A77636) significantly reduce the amplitude of the flash ERG b-wave both under scotopic and photopic conditions of light adaptation. They conclude that the inhibitory effect of dopamine on the ERG, described in rabbit retina, (Jagadeesh & Sanchez, 1981; Textorius, Nilsson, & Andersson, 1989) is most likely mediated through activation of D2 receptors.
and that this action is not specific to the rod or cone pathways in the retina. We could make a similar conclusion for the effect of the endogenous dopamine, acting through D1 receptors, on the frog ERG. However, the relative strength of the dopamine action seems to depend on the photoreceptor input, because SCH 23390 has relatively greater effect on the rod- than cone-mediated suprathreshold b- and d-wave amplitude (the present study). It is generally assumed that the ERG b- and d-waves depend mainly on the activity of ON and OFF bipolar cells, respectively, with minor direct contribution of proximal retinal activity. Our results are in agreement with the results obtained in amphibian retina showing that dopamine, acting through D1 receptors, increased the ratio of amplitudes of the cone-driven to rod-driven components of the bipolar cell responses (Hare & Owen, 1995). Inhibitory action of dopamine on the rod ON bipolar cells, concomitant with a decrease in the amplitude of the b-wave, has been demonstrated also in fish retina (Shiells & Falk, 1985). It is well established that unlike the bipolar cells in mammals, the ON and OFF bipolar cells in amphibian retina receive direct input from both rods and cones (Dowling, 1987). There are no available data concerning the effects of selective D1 receptor agonists and antagonists on the rod- and cone-driven responses of bipolar cells. It has been shown that D1 receptor antagonist SCH 23390 increases the peak amplitude of bipolar cell voltage-gated Na+ currents in bright adapted conditions, suggesting that endogenous dopamine attenuates the sodium channel-dependent amplification of light evoked EPSPs (Ichinose & Lukasiewicz, 2007). We might suggest that the blockade of the above mentioned dopamine action with SCH 23390 will result in an increase of the b-wave amplitude of the photopic ERG, which is consistent with our present results. In our recent paper (Popova & Kupenova, 2010) we reported that the blockade of Na+ currents by TTX caused a decrease of the photopic b-wave amplitude in frog ERG, which supports this suggestion. Some data indicate that SCH 23390 has no apparent action on the rod and cone input to horizontal cells (the other second order retinal neurons), although the selective D1 receptor agonist SKF 38393 attenuates the rod input and increases the cone input (Witkovsky et al., 1988). We might speculate that dopamine, acting through D1 receptors, attenuates to a greater extent the rod input than the cone input to ON and OFF bipolar cells in frog retina.

The process of light adaptation is characterized by changes of the absolute and relative sensitivity as well as in the dynamic range and time course of the retinal responses. Thus, the effects of the D1 receptor blockade on these parameters of the ERG responses give insight into the participation of endogenous dopamine in the process of light adaptation. Our results show that the absolute sensitivity of the dark adapted b-wave is not changed significantly after the D1 receptor blockade. This is consistent with the results of other authors showing that the depletion of retinal dopamine (with 6-OHDA treatment) has no effect on the threshold dark adapted ERG (Li & Dowling, 2000; Lin & Yazzulla, 1994; Naarendorp et al., 1993). But we also demonstrate that the d-wave absolute sensitivity is significantly increased under the influence of SCH 23390. This result supports the suggestion that endogenous dopamine, acting through D1 receptors, contributes to the lower absolute sensitivity of the OFF as compared to the ON response in dark adapted frog ERG (Granut, 1962). Similar result was obtained in our previous study, where the effects of the glycineric and GABAergic blockade on the ERG waves were investigated in dark adapted frog eyes (Popova, 2000). It seems reasonable to argue that a common function of these inhibitory neurotransmitters (glycine, GABA and dopamine acting through D1 receptors) is to suppress threshold scotopic OFF, but not ON response. This suggestion can explain the results of other authors showing that the d-wave diminishes or disappears in the course of dark adaptation (Ren & Lei, 2004). The authors suggest that the observed effect is “likely due to inhibitions from the rod system”. We might propose that this inhibition is mediated through the above mentioned inhibitory neurotransmitters. We obtain that the D1 receptor blockade does not alter significantly also the absolute sensitivity of the photopic b-wave. Thus, it is evident that dopamine action through D1 receptors does not contribute to the existing difference between the absolute sensitivities of the scotopic and photopic ERG ON response. In this aspect the observed effect differs from the effect of the glycineric and GABAergic blockade, which significantly increased the absolute sensitivity of the photopic b-wave (Popova, 2000). We might speculate that the cone-mediated ON responses of the dopaminergic neurons have higher threshold than those of the glycineric and GABAergic retinal neurons. SCH 23390 does not alter or increases significantly the absolute sensitivity of the photopic d-wave depending on the criterion amplitude chosen for its assessment (5 µV or 10 µV). This suggests that the inhibitory action of dopamine (through D1 receptors) develops at lower response amplitude for the photopic d-wave (between 5 and 10 µV) than for the photopic b-wave (>10 µV). Thus a clear ON–OFF asymmetry of the dopamine action on the threshold ERG responses emerges regardless of the state of adaptation.

We obtain that the relative sensitivity of both the scotopic and photopic b-wave is increased after the D1 receptor blockade. Our results are consistent with the results of other authors showing that the enhancing effect of the dopaminergic blockade on the b-wave amplitude was more pronounced at lower stimulus intensities (Oliver et al., 1987; Schneider & Zrenner, 1991). The increased relative sensitivity of the b-wave in our present study is accompanied by a steeper slope and narrowed dynamic range of the response. This result suggests that endogenous dopamine, acting through D1 receptors, is involved in widening of the intensity range, where the b-wave amplitude is a linear function of log I, by preventing saturation to occur at lower stimulus intensity. This might be the contribution of this neurotransmitter to the retinal sensitivity control in frog retina similar to the action of glycine, but not GABA (Popova, 2000). On the other hand, our results demonstrate that SCH 23390 has no significant effect on the relative sensitivity of the d-wave both in dark and light adapted eyes. As a consequence, the difference between the relative sensitivity of the ERG ON and OFF response is further enhanced by the D1 receptor blockade. We are not able to compare this result with results of other authors, because there are no data available about the influence of dopaminergic agents on the amplitude of the d-wave at different stimulus intensities. The described effect of the D1 receptor blockade on the d-wave relative sensitivity differs markedly from the enhancing effect of the glycineric and GABAergic blockade on it (Popova, 2000). This might be due to differences between the intensity-response function of the OFF responses of the dopaminergic neurons, on the one hand, and the glycineric and GABAergic neurons, on the other hand.

It is well established that the rod- and the cone-dominated suprathreshold electroretinograms differ markedly in respect to their b/d amplitude ratio. It has much higher values in the rod- than the cone-dominated ERG. Our present results indicate that dopamine action through D1 receptors could contribute to this phenomenon. We show that the D1 receptor blockade significantly decreases the b/d amplitude ratio in the intensity range, where the responses are mediated by rods and does not alter it in the intensity range, where the responses are mediated by cones. Thus, the initial difference between the b/d ratio values obtained in the rod- and cone-dominated ERG becomes smaller. The described effect of D1 receptor blockade is similar to the effects of the glycineric and GABAergic blockade on frog ERG (Popova, 2000). But the D1 receptor blockade has a specific effect on the b/d amplitude ratio in the intensity range, where transition from rod to cone dominated responses occurs. SCH 23390 significantly increases this ratio, while strychnine and picrotoxin decrease it (Popova, 2000). This
specific effect is due to the lack of dopaminergic inhibition (mediated through D1 receptors) upon the ERG OFF response in this intensity range, while the other two neurotransmitters exert their strongest inhibition. Thus, the first (rod-mediated) and second (cone-dominated) component of the d-wave V-log I curve are separate with a deeper through after D1 receptor blockade and with a shallower one after glycinegic and GABAergic blockade. It seems reasonable to conclude that the specific shape of the dark adapted d-wave V-log I curve depends to some extent on the action of these inhibitory neurotransmitters.

It is well known that adaptation to increased background illumination is characterized by speeding the time course of the light-evoked responses. Our results indicate that dopamine, acting through D1 receptors, does not contribute to this phenomenon for the ERG b- and d-waves. The D1 blockade increases the implicit time of the rod-dominated b-wave, but does not change significantly this of the cone-dominated b-wave. Thus, the difference between the implicit times of the rod- and cone-dominated b-wave is even augmented after the blocker application. The same is true for the d-wave, where SCH 23390 increases to a greater extent the implicit time of the rod- than cone-dominated d-wave.

5. Conclusions

The results of our study clearly show that D1 receptor blockade has similar enhancing effect on the b- and d-wave amplitude irrespective of the state of retinal adaptation and the type of the photoreceptor input. An important conclusion from this study is that endogenous dopamine acting through D1 receptors does not play a crucial role in process of retinal adaptation, although it changes in a specific manner the intensity-response function of both the ERG b- and d-waves. A clear ON–OFF asymmetry of the dopamine action on the absolute and relative sensitivity of the ERG responses is demonstrated.

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References


