Vision Research 101 (2014) 138–150

Contents lists available at ScienceDirect

Vision Research

journal homepage: www.elsevier.com/locate/visres

Effects of picrotoxin on light adapted frog electroretinogram are not due entirely to its action in proximal retina

E. Popova

Department of Physiology, Medical Faculty, Medical University, 1431 Sofia, Bulgaria

ARTICLE INFO

Article history:
Received 3 April 2014
Received in revised form 20 June 2014
Available online 3 July 2014

Keywords:
Electroretinogram
Distal retina
Frog
GABA
Picrotoxin
Proximal retina

ABSTRACT

In order to evaluate the site of action of picrotoxin (antagonist of ionotropic GABA receptors) on the electroretinographic (ERG) b- and d-waves, in this study we compared its effects on the intensity–response function of the ERG waves in intact light adapted frog eyecup preparations with its effects in eyecups, where the activity of proximal neurons was blocked by 1 mM N-methyl-D-aspartate (MNDA). Picrotoxin markedly enhanced the b- and d-wave amplitude and slowed the time course of the responses at all stimulus intensities in the intact eyecups. Perfusion with NMDA alone caused significant enhancement of the b-wave amplitude and diminution of the d-wave amplitude without altering their time course in the entire intensity range. When picrotoxin was applied in combination with NMDA, an enhancement of the b-wave amplitude and slowing of its time course were observed at all stimulus intensities. The increase of the b-wave amplitude was significantly higher than that seen in NMDA group. Combined application of picrotoxin and NMDA caused an enhancement of the d-wave amplitude at the lower stimulus intensities and its diminution at the higher ones, while the d-wave time course was delayed over the entire intensity range. The results obtained indicate that a part of picrotoxin effects on the amplitude and time course of the photopic ERG b- and d-waves are due to its action in the distal frog retina.

1. Introduction

GABA is the major inhibitory neurotransmitter in the vertebrate retina, released mainly from two types of interneurons – GABAergic horizontal cells and GABAergic amacrine cells (reviews: Eggers et al., 2006; Wu & Maple, 1998; Yang, 2004; Yazulla, 1986). The GABAergic horizontal cells modulate the synaptic transmission at the level of the outer plexiform layer (OPL), while the GABAergic amacrine cells do this in the inner plexiform layer (IPL). GABA, released in the both plexiform layers, can modulate the activity of bipolar cells, which transmit visual information from photoreceptors to the output neurons of the retina. GABA, released in the OPL can modulate bipolar cell activity by two types of inhibition. The first one is feed-forward inhibition accomplished by GABA acting directly on $GABA_A$ and $GABA_C$ receptors expressed on bipolar cell dendrites (Connaughton, Nelson, & Bender, 2008; Du & Yang, 2000; Euler & Wässle, 1998; Grünert, 2000; Kalloniatis & Tomisich, 1999; Lukasiewicz & Shields, 1998; Vardi et al., 1998; Vitanova et al., 2001; Wässle et al., 1998). The second one is feedback inhibition carried out by GABA acting on $GABA_A$ and $GABA_C$ receptors located on cone terminals (Kaneko & Tachibana, 1986; Lin & Yazulla, 1994; Pattnaik et al., 2000; Picaud et al., 1998; Vardi, Masarachia, & Sterling, 1992; Yang, Lin, & Yazulla, 1992; Yazulla & Studholme, 1997). GABA, released in the IPL can exert feedback inhibition upon the bipolar cell axon terminals, which possess both $GABA_A$ and $GABA_C$ receptors (Enz et al., 1996; Fletcher, Koulen, & Wässle, 1998; Koulen et al., 1998; Lukasiewicz & Wong, 1997; Shields et al., 2000; Wässle et al., 1998). The $GABA_A$ receptors mediate the phasic component, while GABAc receptors mediate the tonic component of the response to GABA (Eggers & Lukasiewicz, 2011; Herrmann et al., 2011; Vigh & von Gersdorff, 2005; review: Lukasiewicz et al., 2004). The relative contribution of each type of the GABAergic interneurons to the overall GABA effect upon the amplitude and temporal characteristics of the bipolar cell light responses remains poorly understood.

One of the easiest ways to characterize light responses of all groups of bipolar cells *in vivo* without perturbing any neuronal connections is by recording electroretinogram (ERG). The 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). There is general consensus that the neuronal generator of the b-wave is primarily the depolarizing (ON) bipolar cells (Dick & Miller, 1978, 1985; Gargini et al., 1999; Green & Kapousta-Bruneau, 1999; Gurevich & Slaughter, 1993; Hanitzsch, Lichtenberger, & Mattig, 1996; Karwoski & Xu, 1999; Newman & Odette, 1984; Robson &

E-mail address: epopova@abv.bg

http://dx.doi.org/10.1016/j.visres.2014.06.012
0042-6989/

Frishman, 1995; Shiells & Falk, 1999; Sieving, Murayama, & Naarendorp, 1994; Tian & Slaughter, 1995; Xu & Karwoski, 1994; Yanagida et al., 1986). The d-wave generation is thought to depend mainly on the activity of hyperpolarizing (OFF) bipolar cells with minor contribution of the photoreceptor response at stimulus offset (Dick, Miller, & Daucheux, 1979; Stockton & Slaughter, 1989; Ueno et al., 2006; Xu & Karwoski, 1995; Yanagida et al., 1986) and activity of proximal retinal neurons (*amphibians*: Arnarsson & Eysteinsson, 1997, 2000; Awatramani, Wang, & Slaughter, 2001; Popova & Kupenova, 2009). Thus, the role played by endogenous GABA in shaping the response characteristics of large populations of ON and OFF bipolar cells could be easily investigated by exploring the effects of simultaneous $GABA_A$ and $GABA_C$ receptor blockade on the ERG b- and d-waves. Unfortunately, the results obtained in such studies are contradictory. In *mammalian retina* Kapousta-Bruneau (2000) has reported that picrotoxin ($GABA_A$ and $GABA_C$ receptor antagonist) has no effect on the dark adapted b-wave amplitude in rat ERG. The authors cited have found that the $GABA_A$ receptor antagonist bicuculline increases the b-wave amplitude, while the $GABA_C$ receptor antagonist 3-APA reduces the amplitude of the b-wave. Thus, the action of $GABA_A$ and $GABA_C$ antagonists cancels out each other. Other authors, however, have demonstrated that picrotoxin decreases the amplitude and selectively prolongs the decay, but not the rise time of the dark adapted b-wave (*rabbit*: Dong & Hare, 2002; Starr, 1975; *cat*: Naarendorp & Sieving, 1991). The effect does not depend on the stimulus intensity and is evident over the entire intensity range studied (Naarendorp & Sieving, 1991), although the effect on the b-wave kinetics appears to be greater at lower intensities (Dong & Hare, 2002). Still other authors reported that the b-wave amplitude is increased and its time course is delayed under the influence of picrotoxin in dark adapted rabbit retina (Gottlob, Wündsch, & Tuppy, 1988). There are no available data concerning the effects of picrotoxin on the mammalian d-wave.

The effects of picrotoxin on the ERG waves have been more extensively studied in *nonmammalian retina*. Some authors fail to obtain any effect of picrotoxin on the amplitude of the b-wave (*tiger salamander*: Wachtmeister, 1980), while other authors reported that picrotoxin reduces the b-wave substantially at all flash intensities in conditions of dark adaptation (*fish*: Chappell et al., 2002). Chappell et al. (2002) have demonstrated that the blocker has no effect on the light responses of horizontal cells recorded simultaneously with the ERG. This finding support the suggestion that photoreceptor feedback is not involved in picrotoxin effect on the ERG. The authors concluded that picrotoxin-induced reduction in the b-wave results from inactivation of the $GABA_C$ receptors of ON bipolar cells. However, many other data including those obtained in our laboratory indicate that the blockade of ionotropic GABA receptors by picrotoxin leads to an increase of the b-wave amplitude (*frog*: Arnarsson & Eysteinsson, 1997; Belcheva & Kupenova, 1980; Belcheva & Vitanova, 1974; Bonaventure, Wioland, & Jardon, 1986; DeVries & Friedman, 1978; Katz et al., 1991; Kupenova, Popova, & Vitanova, 2008; Popova, 1989, 2000; Popova et al., 1986; *turtle*: Belcheva & Vitanova, 1978; Kupenova et al., 1991, 1997; Vitanova et al., 1997; *fish*: Chappell & Rosenstein, 1996; Lewis et al., 2011). The effect is evident in conditions of both dark and light adaptation, indicating that it does not depend on the type of the photoreceptor input. Picrotoxin slows the time course of the b-wave. Its latency, implicit time and decay phase are significantly delayed during the GABAergic blockade (Belcheva & Vitanova, 1974, 1978; Popova, 2000; Vitanova et al., 2001). The effect on the latency is considerably smaller compared to the effect on the implicit time (Popova, 2000). The effects of picrotoxin on the d-wave resembled that on the b-wave. Although Bonaventure, Wioland, and Jardon (1986) did not find any effect of picrotoxin on the d-wave, other authors reported that picrotoxin enhances the amplitude and delays the time course of the d-wave (Arnarsson & Eysteinsson, 1997; Belcheva & Kupenova, 1980; Belcheva & Vitanova, 1974, 1978; Kupenova et al., 1991, 1997; Lewis et al., 2011; Popova, 1989, 2000; Popova et al., 1986; Vitanova et al., 1997; Xu & Karwoski, 1995). Picrotoxin revealed a prominent OFF component in all rod retina of skate (Chappell & Rosenstein, 1996) and in dark adapted toad retina (Katz et al., 1991). Thus, it appears that both the ON and OFF responses in nonmammalian ERG are under inhibitory GABAergic influences mediated by ionotropic GABA receptors.

Most of the authors believe that the observed GABAergic influences on the ERG b- and d-waves occur only in the proximal retina. It has been shown that the enhancement of the b- and d-wave amplitude caused by picrotoxin in light adapted Xenopus retina is entirely eliminated, when the activity of proximal retinal neurons is blocked by high doses of glutamate agonist N-methyl-D-aspartate (NMDA) (Arnarsson & Eysteinsson, 1997). The same has been observed for the dark adapted b-wave in toad retina (Katz et al., 1991). Katz et al. (1991) have shown that picrotoxin markedly increases the amplitude of the b-wave. The subsequent application of NMDA subtracts from the picrotoxin response nearly everything that the blocker had added to the response. Analogous is NMDA effect on the proximal K^+ increase – it completely abolishes the enhancing effect of picrotoxin upon it. The authors suggest that the effects of picrotoxin are due to its action in proximal retina, where it inhibits the amacrine cell-mediated reciprocal inhibition of bipolar cells. Using current-source density analysis of the amphibian and rabbit ERG, Karwoski, Xu, and Yu (1996) and Karwoski and Xu (1999) concluded that picrotoxin enhances the proximal field potentials, whereas the blocker little affects the b- and d-wave sources. The authors cited have found that picrotoxin greatly enhances the M-wave, which arises from Muller cells through the spatial buffering of the light-evoked K^+ increase in the inner plexiform layer. The M-wave is generally documented in the intraretinal or "local" ERG and is not typically recorded in the diffuse transretinal ERG. Similar effect of picrotoxin has been reported in light adapted cat retina, where the blocker greatly enhances both the ON and OFF portions of the M-wave, but it does not affect the negative ERG OFF response (Frishman & Steinberg, 1990). All these results support the hypothesis that the effects of picrotoxin on the b- and d-waves are due primarily to its action in proximal retina. However, Chappell and Rosenstein (1996) have shown that the enhanced ON and OFF components of skate ERG recorded during picrotoxin treatment are not reduced during the subsequent application of 500 μM NMDA. They proposed that picrotoxin effect is due to release from inhibition at the distal dendrites of ON and OFF bipolar cells. Kapousta-Bruneau (2000) also argue that the proximal neurons containing NMDA receptors are not involved in the modulatory GABA effects on the dark adapted b-wave in rat ERG (Kapousta-Bruneau, 2000). The author has shown that suppression of NMDA receptors with 25 μM MK-801 eliminates neither the enhancing effect of bicuculline ($GABA_A$ receptor antagonist) nor the depressing effect of 3-APA (GABAc receptor antagonist) on the $P_2(t)$ component of ERG. Surprisingly, suppression of all types of ionotorpic glutamate receptors (NMDA and KA/AMPA) expressed on GABAergic amacrine and GABAergic horizontal cells also does not eliminate the effect of bicuculline on $P_2(t)$ component of ERG. The author cannot elucidate which is the source of GABA in this situation, when the activity of all GABAergic neurons in the retina is suppressed.

In the present study we compared the effects of picrotoxin on the intensity–response function of the ERG b- and d-waves in intact light adapted frog eyecup preparations with its effects in eyecups, where the activity of proximal retinal neurons was blocked by 1 mM NMDA. We obtained that picrotoxin markedly

enhanced the b- and d-wave amplitude and slowed the time course of the responses at all stimulus intensities in the intact eyecups. The blocker's effects on the b-wave were preserved in some extent over the entire intensity range in eyecups treated with NMDA. The same was true for the picrotoxin effects on the d-wave time characteristics, while its action on the d-wave amplitude depended on stimulus intensity. Picrotoxin enhanced the d-wave amplitude at the lower stimulus intensities, but diminished it at the higher ones. The results obtained indicate that a part of picrotoxin effects on the amplitude and time course of the photopic ERG b- and d-waves are due to its action in the distal frog retina.

2. Material and methods

The experiments were carried out on 46 eyecup preparations of frog (*Rana ridibunda*), continuously superfused with Ringer solution (NaCl 110 mM, KCl 2.6 mM, $NaHCO_3$ 10 mM, $CaCl_2$ 1.6 mM, $MgCl_2$ 0.8 mM, Glucose 2 mM; HCl 0.5 mM to adjust pH to 7.8) at a rate of 1.8–2.0 ml/min, temperature 16–18 °C and supplied with moistened O_2 (for details see Popova & Kupenova, 2009). The frogs were first anesthetized in water containing 500 mg/l Tricaine methanesulfonate (Sigma–Aldrich Chemie GmbH). They were then decapitated and pithed. The procedure has been approved by the local ethics committee and is in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

The ionotropic GABA receptors were blocked using 50 μM picrotoxin (Flika, Buchs, Switzerland) dissolved in the perfusion solution. This concentration was chosen among the other tested because it had maximal effect on both the b- and d-wave amplitude (Popova, 2000). The activity of proximal neurons was blocked using N-methyl-D-aspartate (NMDA – Sigma), dissolved in Ringer solution to a concentration of 1 mM. The same concentration has been used in our previous study (Popova & Kupenova, 2009) and by other authors working on amphibian retina (Arnarsson & Eysteinsson, 1997, 2000; Awatramani, Wang, & Slaughter, 2001). The saturating nature of this NMDA concentration was tested in some eyecups, where a higher concentration of NMDA (2 mM) was applied.

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_t) was changed in an ascending manner over a range of 5 log units by means of neutral density filters. Stimulus intensities were measured using radiometer. They were then converted in quanta s^{-1} μm^{-2}, falling at the plane of the retina. The maximal intensity (denoted by 0) was 6×10^8 quanta s^{-1} μm^{-2} or 5×10^4 lx. The following equation was used to convert between the two units of measurement: N (quanta s^{-1} m^{-2}) = $k \times B$; $k = 0.735 \times 10^{22} \frac{\sum E \cdot \lambda}{\sum E \cdot S}$, where B, illumination (lx); λ, wavelength (m); E, relative emission of the source at each wavelength (3200 K); S, relative sensitivity of the photometer at each wavelength. The test stimuli were presented under diffuse white background illumination with intensity of 2.4×10^6 quanta s^{-1} μm^{-2} or 6.6×10^4 quanta s^{-1} μm^{-2} at 500 nm, which was sufficient to saturate the rods (Fain, 1976; Hood & Hock, 1975). The type of the photoreceptor input was proved in our previous work by ERG response spectral sensitivity assessment (Popova & Kupenova, 2011).

2.2. Experimental procedure

The frogs were dark adapted for 24 h and then the eyecup preparation was made under dim red light. The eyecups were adapted under photopic background for 15 min and then the V–log$I$ function of the ERG waves was obtained using stimuli with increasing intensity (first series). After a new 15 min adaptation period, a 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 50 μM picrotoxin alone, 1 mM NMDA alone or combination of 50 μM picrotoxin and 1 mM NMDA. The perfusion was switched from Ringer solution to 50 μM picrotoxin 15 min before the beginning of the second intensity series, when the effect of the blocker was fully developed. This period was 10 min for experiments with 1 mM NMDA, because its effects developed faster than that of picrotoxin. In experiments, where the effects of combined application of PT and NMDA were tested, the eyecups were perfused 5 min with 50 μM picrotoxin and afterwards 10 min with combination of 50 μM picrotoxin and 1 mM NMDA during the adaptation period between the first and second intensity series. In additional experiments the effects of two different concentrations (1 mM and 2 mM) of NMDA (Ringer solution in controls resp.) on the ERG waves were followed for a period of 22 min using stimulus with constant intensity ($\log I_t = -3.0$). The chosen stimulus intensity fell in the steepest part of the V–log$I$ function of both the b- and d-waves.

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. The amplitude of the b-wave was measured from the peak of the a-wave to the peak of the b-wave, while that of the d-wave was measured from the baseline to the peak of the wave (see Fig. 1c). The latency of the ERG waves 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 (see Fig. 1c). For estimation 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 5 μV and 10 μV criterion response amplitude. The b-wave V–log$I$ function was fitted to the Naka–Rushton equation: $V = V_{max} \times I^n/(I_\sigma^n + I^n)$, where V, amplitude of the ERG waves; V_{max}, its maximum; 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 & Rushton, 1966). The value of I_σ 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_{max} amplitude. The V–log$I$ function of the d-wave was estimated by smoothing the experimental data using Inductive Algorithm for Smooth Approximation of Functions (IASAF), based on the Tikhonov regularization method and the principle of heuristic self-organization (Kupenova, 2011). The fitting curves passed within the noise limits of the data. The threshold intensity, V_{max} and I_t, producing $0.5V_{max}(I_\sigma)$, were evaluated from the approximating curves. The complex character of the d-wave V–log$I$ function, which consisted of ascendant and descendent parts, did not allow us to determine its dynamic range.

For statistical evaluation of the data, Student's t-test and Two-Way ANOVA were used (OriginPro 8 software, OriginLab Corporation, Northampton, MA).

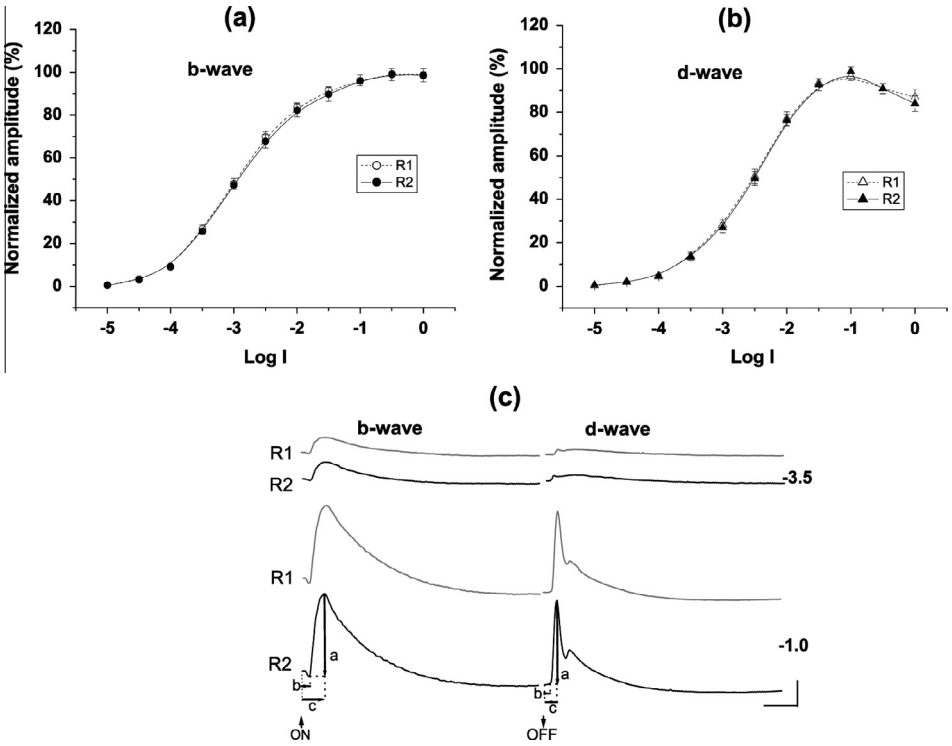

Fig. 1. (a and b) V–log$I$ function of the b- and d-waves, obtained in the control experiments (n = 10). The amplitudes of the ERG waves are normalized to V_{max} of the responses obtained during the first stimulus series of the experiments. The symbols representing the responses obtained during the first (open symbols) and second (filled symbols) intensity series are denoted in the legends. Mean values ± SEM are shown. (c) Original ERG records, obtained with different stimulus intensities during the first (R1) and second (R2) intensity series in the control experiments. The numbers on the right side indicate stimulus intensity (logI_t). With arrows is shown how the amplitude (a), latency (b) and implicit time (c) of the ERG waves were measured. On the bottom of the figure is indicated the moment, when the light stimulus was turn on (ON, upward arrow) and turn off (OFF, (downward arrow). Calibration: time – 500 ms, amplitude – 200 μV.

3. Results

3.1. Control experiments

The V–log$I$ function of the b-wave differed from that of the d-wave at the highest stimulus intensities, where its amplitude plateaued, while that of the d-wave decreased (Fig. 1a and b). The V–log$I$ function of the both waves showed no significant differences between the first and second intensity series in one and the same eyecup (Fig. 1a and b). The absolute sensitivity of the responses (determined by their thresholds) and their relative sensitivity (determined by I_σ value) were similar in both intensity series. The same was true for the dynamic range of the b-wave and the time course of the responses (Fig. 1c). This allowed us to evaluate the effects of substances tested on these parameters using the first series of the test experiments as a control one.

3.2. Effects of picrotoxin on the intensity–response function

Perfusion with 50 μM picrotoxin (PT) caused marked increase of the b- and d-wave amplitude at all stimulus intensities used (Fig. 2a and b). The amplitude of the ERG waves recovered to a great degree during reperfusion with Ringer solution (Fig. 2c). The relative change of the b- and d-wave amplitude during picrotoxin treatment was significantly greater than that in the control experiments in the whole intensity range (Fig. 2d and e) (Two-Way ANOVA, $p < 0.0001$). The absolute sensitivity of the ON and OFF responses was significantly increased, which was evident from the lowered threshold values (Table 1). The d-wave threshold diminished to a greater extent than that of the b-wave (paired t-test $p < 0.0033$ for 5 μV threshold; $p < 0.0002$ for the 10 μV threshold). Thus, the absolute sensitivity of the OFF response became higher as compared to that of the ON response (paired t-test, $p < 0.0033$ for the 5 μV threshold; $p < 0.0016$ for the 10 μV threshold). The relative strength of the PT enhancing effect on the b- and d-wave amplitude depended on stimulus intensity. Two-Way ANOVA revealed significant interaction between the relative amplitude change and stimulus intensity ($p < 0.01$ for the b-wave, $p < 0.0001$ for the d-wave). The relative increase of the b-wave amplitude was maximal near I_δ point (I_t −3.5 and I_t −3.0), while that of the d-wave was expressed to a greater degree in the lower than upper half of the intensity range (Fig. 2d and e). The described dependence of PT effect on stimulus intensity led to left shift of the V–log$I$ curves along the intensity axis and increased relative sensitivity (determined by I_δ value) of the responses (paired t-test, $p < 0.0019$ for the b-wave; $p < 0.0041$ for the d-wave) (Fig. 3a and b). Because the relative sensitivity of the OFF response was increased to a greater extent (with 0.39 ± 0.09 log units) as compared to that of the ON response (with 0.21 ± 0.04 log units; paired t-test, $p < 0.04$), the initial difference between them became smaller. A greater enhancement of the d-wave than b-wave amplitude was seen at all lower stimulus intensities ($I_t < -3$), which accounted for the significantly lower b/d amplitude ratio at those intensities (Two-Way ANOVA, $p < 0.0001$) (Fig. 2f). The opposite was true for the higher intensity range, where PT had stronger effect on the b-wave than d-wave amplitude and the b/d amplitude ratio was significantly increased (Two-Way ANOVA, $p < 0.0001$). Thus, the b/d amplitude ratio obtained with different photopic stimulus intensities, depends in a critical manner on the relative strength of the GABAergic inhibitory influences upon the ON and OFF channel. Perfusion with PT alone did not change significantly the slope of the b-wave V–log$I$ function and its dynamic range (Fig. 3a).

The GABAergic blockade slowed the time course of the ERG waves (Fig. 3c–e). The latency of the b- and d-waves was significantly delayed during the perfusion with PT at all stimulus

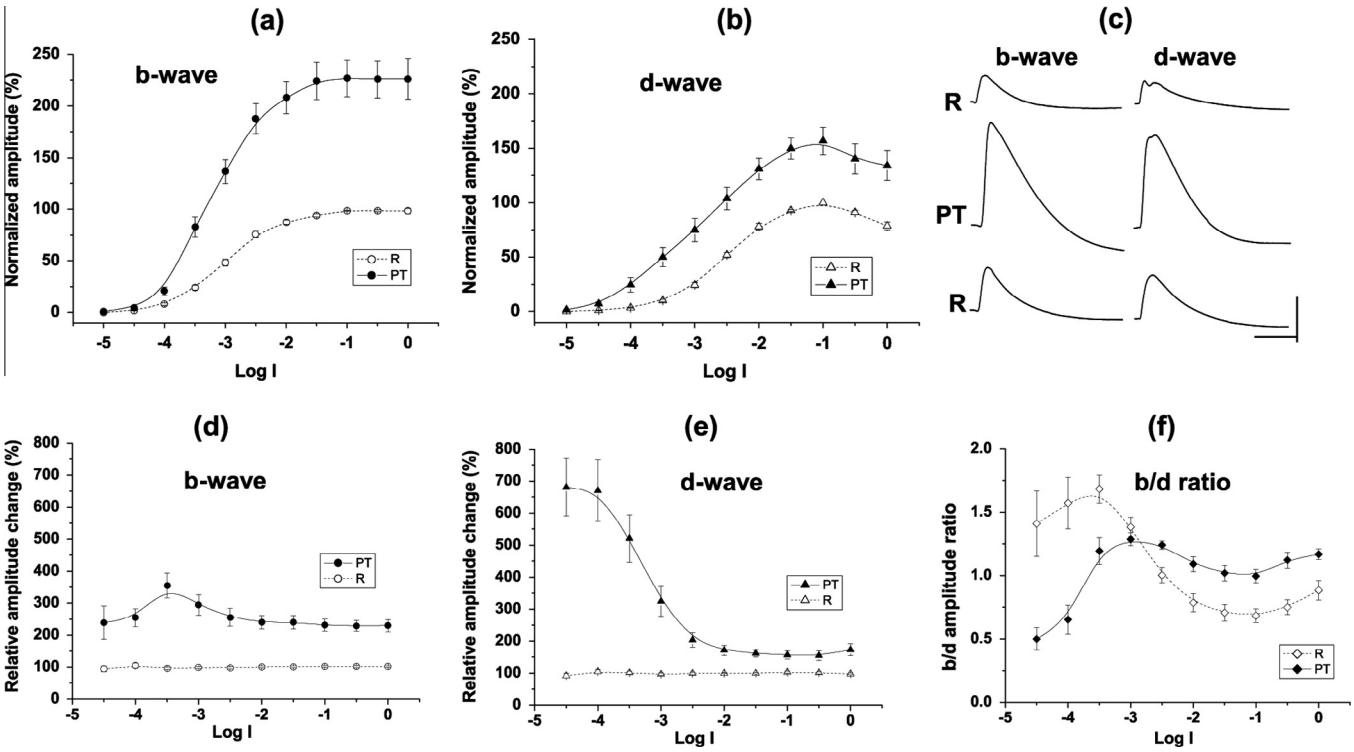

Fig. 2. (a and b) Effects of picrotoxin on the V–log$I$ function of the ERG b- and d-waves. The amplitudes of the ERG waves are normalized to V_{max} of the responses obtained during the first series of the experiments. The symbols representing the responses obtained during the first (open symbols) and second (filled symbols) intensity series are denoted in the legends. Mean values ± SEM are shown (n = 8). (c) Original ERG records (b- and d-wave), obtained during the perfusion with Ringer in the control period (upper row), picrotoxin (middle row) and Ringer in the recovery period (bottom row). Calibration: time – 500 ms; amplitude – 100 μV. (d and e) Relative amplitude change of the ERG b-wave (c) and d-wave (d) in the control experiments (open symbols) and picrotoxin experiments (filled symbols). The amplitudes of the ERG waves obtained at each I_t during the second intensity series were normalized to the ERG amplitudes obtained during the first series. Mean values ± SEM are shown. (e) Changes in the b/d amplitude ratio in the first (open symbols) and second (filled symbols) intensity series in picrotoxin experiments. Means ± SEM are represented.

Table 1
Effects of picrotoxin, NMDA and combination of picrotoxin + NMDA on the threshold values of the ERG b- and d-waves.

ERG wave	Threshold (log$I$)	
	5 μV	10 μV
b-Wave		
Before PT	−4.52 ± 0.08	−4.29 ± 0.10
After PT	−4.74 ± 0.10	−4.58 ± 0.11
	p < 0.0043	p < 0.0012
d-Wave		
Before PT	−4.44 ± 0.09	−4.15 ± 0.10
After PT	−5.01 ± 0.09	−4.82 ± 0.10
(n = 8)	p < 0.0001	p < 0.0001
b-Wave		
Before NMDA	−4.51 ± 0.09	−4.24 ± 0.10
After NMDA	−4.50 ± 0.08	−4.31 ± 0.09
		p < 0.024
d-Wave		
Before NMDA	−4.52 ± 0.10	−4.22 ± 0.12
After NMDA	−4.14 ± 0.10	−3.86 ± 0.11
(n = 15)	p < 0.0001	p < 0.0001
b-Wave		
Before NMDA + PT	−4.63 ± 0.12	−4.39 ± 0.09
After NMDA + PT	−4.69 ± 0.08	−4.51 ± 0.08
		p < 0.009
d-Wave		
Before NMDA + PT	−4.56 ± 0.06	−4.31 ± 0.06
After NMDA + PT	−4.72 ± 0.07	−4.50 ± 0.06
(n = 9)	p < 0.031	p < 0.002

intensities (Two-Way ANOVA, p < 0.0001). The same was true for their implicit time (Two-Way ANOVA, p < 0.0001). The effect on the implicit time was considerably bigger compared to the effect on the latency (Fig. 3c and d) and it showed significant dependence on stimulus intensity (Two-Way ANOVA, p < 0.0078 for the b-wave; p < 0.0001 for the d-wave). It was expressed to a greater extent at the lower (I_t < −2.5) than higher stimulus intensities for the both ERG waves. Perfusion with PT reversed the initial relationship between the implicit times of the b- and d-wave obtained in the lower intensity range (I_t < −2.5). In control conditions the d-wave implicit time was shorter than that of the b-wave in that intensity range (Two-Way ANOVA, p < 0.0001), while after the GABAergic blockade it became longer (Two-Way ANOVA, p < 0.0043) (Fig. 3c and d). The latter effect was due to the greater change of the d- than b-wave implicit time during PT treatment.

3.3. Effects of NMDA on the intensity–response function

Before studying the effects of 1 mM NMDA on the intensity–response function, we did additional experiments with constant stimulus intensity (logI_t = −3.0) in order to test if this concentration of NMDA was a saturating one. In our previous study (Popova & Kupenova, 2009) we have shown that application of 1 mM NMDA caused marked increase of the b-wave amplitude and diminution of the d-wave amplitude (Fig. 4a and b). The effect on the b-wave reached a plateau at the 10th minute from the beginning of NMDA perfusion, while that on the d-wave developed much faster. The NMDA 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. 4c). In another group of experiments the eyecups were treated with 1 mM NMDA first and with 2 mM NMDA afterwards (Fig. 4a and b). The perfusion was switched from the

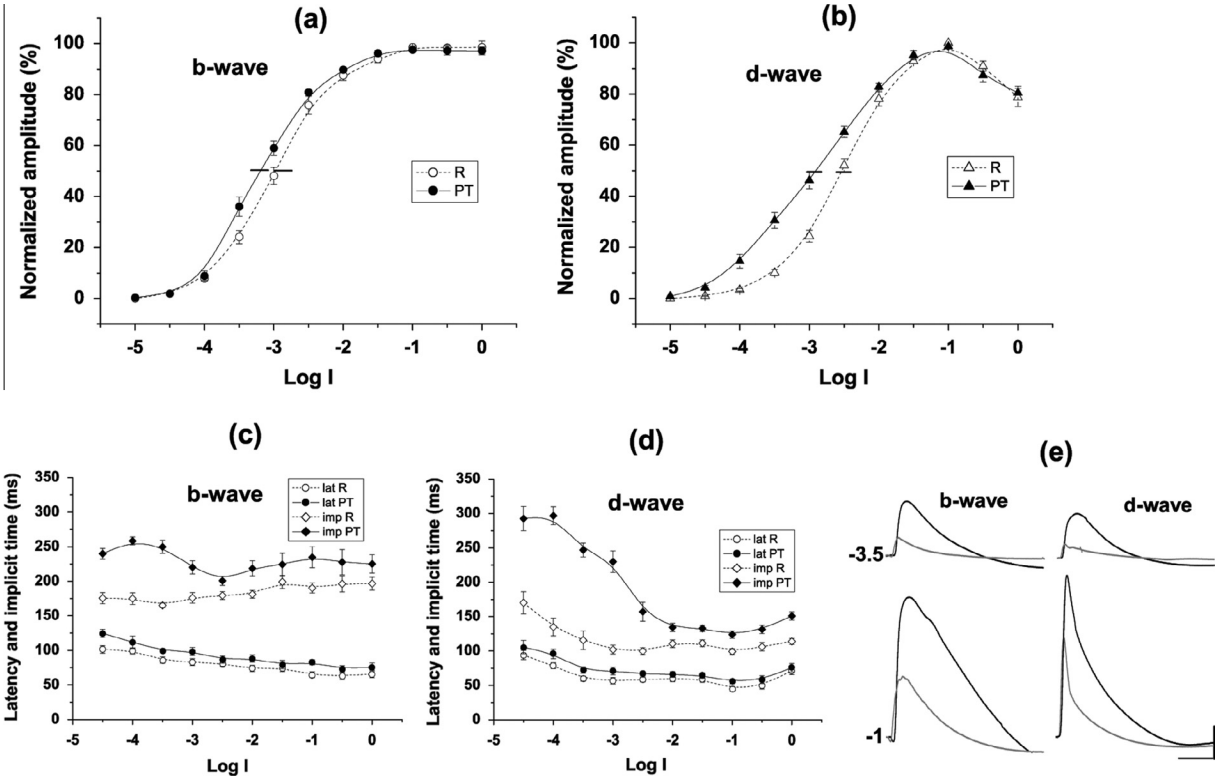

Fig. 3. (a and b) Normalized V–log$I$ curves of the b- and d-waves obtained during the first (open symbols) and the second (filled symbols) series of the picrotoxin experiments. The values in each series are normalized to the V_{max} obtained in the same series. This manner of representation demonstrates the changes of the position of the V–log$I$ function along the intensity axis and its steepness under the influence of picrotoxin. The I_σ point is denoted with a black horizontal line on the curve. (c and d) Changes of the latency (lat) and implicit time (imp) in the first (open symbols) and second (filled symbols) intensity series in picrotoxin experiments. Means ± SEM are represented. (e) Original ERG records, obtained with different stimulus intensities during the control period (grey lines) and during the treatment with picrotoxin (black lines). The numbers on the left side indicate stimulus intensity (logI_t). Calibration: time – 500 ms, amplitude – 200 μV.

lower to the higher NMDA concentration in time period, when the effect of the blocker on the ERG waves was fully developed (arrow at 17th minute). No additional effect of 2 mM NMDA on the b- and d-wave amplitude was seen. However, the effect of 2 mM NMDA was not very stable in time and a tendency for diminution of the ERG wave's amplitude was evident. We chose concentration of 1 mM NMDA for the main groups of experiments, because it was important to obtain the V–log$I$ function of the ERG waves on the background of stable blocker's effect.

Perfusion with 1 mM NMDA had opposite effects on the amplitude of the b- and d-waves over the entire intensity range studied. It caused a significant enhancement of the b-wave amplitude and a significant diminution of the d-wave amplitude at all stimulus intensities (Two-Way ANOVA, $p < 0.0001$ for the both waves) (Fig. 5a and b). As a result of this, the b/d amplitude ratio was significantly increased over the entire range of stimulus intensities (Two-Way ANOVA, $p < 0.0001$) (Fig. 5c). The 5 μV threshold of the b-wave was not significantly altered, while its 10 μV threshold was significantly lowered (Table 1). This indicates that the enhancing effect of NMDA upon the ON response started at stimulus intensities, producing b-wave amplitude between 5 μV and 10 μV. On the other hand, both the 5 μV and 10 μV thresholds of the d-wave were significantly increased, showing that the suppressing effect of NMDA upon the OFF response is evident even at stimulus intensities eliciting d-wave amplitude ⩽5 μV. The relative amplitude change of the b-wave, cause by NMDA, did not depend on stimulus intensity, while that of the d-wave showed a clear intensity dependence (Two-Way ANOVA, $p < 0.0089$) (Fig. 5d and e). It was greatest at the very low stimulus intensities and diminished with increasing stimulus intensity. In consequence of the described effects, the relative sensitivity of the d-wave (determined with I_δ point) was significantly decreased (paired t-test, $p < 0.002$), while that of the b-wave remained unchanged during the blockade of proximal retinal activity. The dynamic range of the b-wave V–log$I$ function also remained unchanged during the NMDA treatment. The same was true for the time characteristics of the ERG waves. Neither the latency, not the implicit time of the b- and d-waves were significantly altered during the perfusion with NMDA (Fig. 5f). Similar results were obtained in our previous study, where the effects of NMDA were tested in light adapted frog eyecups (Popova & Kupenova, 2009).

3.4. Effects of combination of picrotoxin and NMDA on the intensity–response function

The effects of perfusion with combination of PT and NMDA showed clear ON/OFF asymmetry. The b-wave amplitude was enhanced at all stimulus intensities (except for the lowest ones) (Two-Way ANOVA, $p < 0.0001$), while that of the d-wave was enhanced in the lower half of the intensity range (Two-Way ANOVA, $p < 0.0001$), but diminished in the upper half (Two-Way ANOVA, $p < 0.0001$) (Fig. 6a and b). The 5 μV threshold of the b-wave was not significantly changed, while its 10 μV threshold was significantly lowered (Table 1). This indicates that the potentiating effect of PT upon the ON response was not developed at the lowest stimulus intensities (producing b-wave amplitudes ⩽5 μV) during the blockade of proximal retinal activity. This effect started at stimulus intensities producing b-wave amplitude between 5 μV and 10 μV. On the other hand, both the 5 μV and 10 μV thresholds of the d-wave were significantly lowed (Table 1),

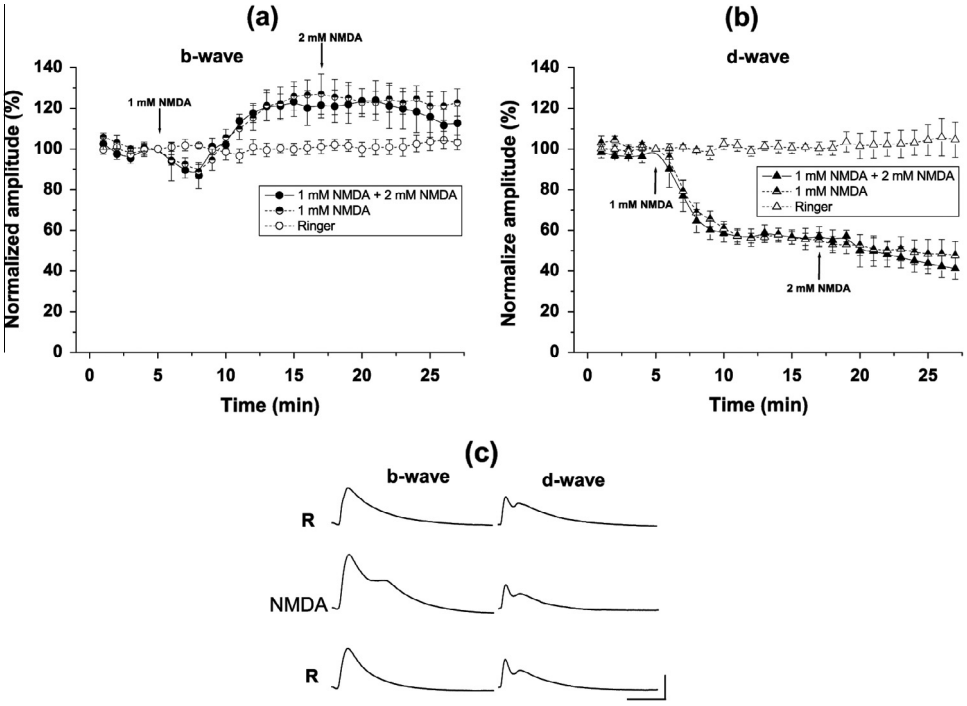

Fig. 4. (a and b) Time-course of the NMDA effect on the amplitude of the b-wave (left) and d-wave (right), obtained with $\log I_t = -3.0$. The amplitudes of the ERG waves are normalized to the values obtained just prior to NMDA treatment. The times, when the perfusion was switched to 1 mM NMDA or 2 mM NMDA, are indicated by arrows. Mean values ± SEM are shown. The symbols represented the responses obtained in the group with application of 1 mM NMDA ($n = 6$), group with application of 1 mM NMDA first and 2 mM NMDA afterwards ($n = 4$) and control group with Ringer solution perfusion only ($n = 6$) are denoted in the legends. (c) Original ERG records (b- and d-wave), obtained during the perfusion with Ringer in the control period (upper row), 1 mM NMDA (middle row) and Ringer in the recovery period (bottom row). Calibration: time – 500 ms; amplitude – 200 μV.

showing that the blockade of the proximal retinal activity did not prevent the enhancing effect of picrotoxin on the absolute sensitivity of the ERG OFF response. The relative increase of the d-wave amplitude, obtained in the lower half of the intensity range, showed a clear dependence on stimulus intensity (Two-Way ANOVA, $p < 0.0006$). It was greatest at the lowest suprathreshold intensities (I_t −4 and −3.5) and decreased toward the higher ones (Fig. 6d). The relative increase of the b-wave amplitude was maximal at stimulus intensities near and below I_δ point (I_t from −4 to −3) (Fig. 6c), which led to a left shift of the b-wave V–log function along the intensity axis and a decreased value of the I_δ point (paired t-test, $p < 0.041$) (Fig. 7a). The latter result indicates that the enhancing effect of PT on the relative sensitivity of the ERG ON response was preserved on the background of proximal retinal blockade. The same was true for the effect of picrotoxin on the relative sensitivity of the ERG OFF response. Because the d-wave amplitude was increased in the lower half of the intensity range, but diminished in the upper half, the V–log function of the d-wave was shifted to the left along the intensity axis and its relative sensitivity (determined by I_δ value) was greatly increased (paired t-test, $p < 0.00007$) (Fig. 7b). The effect was expressed in nearly the same extent as that seen in picrotoxin group (compare Figs. 3b and 7b), demonstrating that the effect of the GABAergic blockade on the relative sensitivity of the photopic OFF response does not depend critically on the activity of proximal retinal neurons. The initial difference between the relative sensitivity of the ON and OFF response was greatly diminished during the combined application of PT and NMDA. This was due to significantly greater change of the relative sensitivity of the OFF response as compare to that of the ON response (paired t-test, $p < 0.0062$). The same effect was seen in experiments, where picrotoxin was applied alone. Perfusion with PT + NMDA altered the b/d amplitude ratio in a predictable way. Because each of the blockers increased the b/d ratio in the higher intensity range, their combined application caused marked increase of the b/d amplitude ratio at all stimulus intensities higher than −3.5 (Two-Way ANOVA, $p < 0.0001$) (Fig. 6e). On the other hand, because the two blockers had opposite effects on the b/d ratio in the lower intensity range, it magnitude did not changed significantly in that range. Perfusion with PT + NMDA did not alter the slope of the b-wave V–log$I$ function and its dynamic range (Fig. 7a). This result could be expected, because neither PT nor NMDA alone changed them.

Combined application of PT and NMDA slowed the time course of the b- and d-waves. The latency of the b- and d-waves was significantly delayed (Two-Way ANOVA, $p < 0.0039$ for the b-wave; $p < 0.0001$ for the d-wave) as well as their implicit time (Two-Way ANOVA, $p < 0.0001$) over the entire intensity range (Fig. 7c–e). The effect on the latency was less pronounced compared to the effect on the implicit time. The observed effects on the time characteristics of the responses were probably due to the action of picrotoxin and not NMDA, because perfusion with NMDA alone did not change the time course of the ERG waves. Thus, it can be hypothesized that the action of picrotoxin in the distal retina results in delaying the time course of the photopic ERG waves. The effect of PT + NMDA on the d-wave implicit time depended on stimulus intensity (Two-Way ANOVA, $p < 0.0001$). It was expressed to a bigger extent at the lower ($I_t < -2.5$) stimulus intensities (Fig. 7d and e). The prolongation of the d-wave implicit time was relatively larger compared to the b-wave in the lower intensity range, which led to a reversal of the relationship between them in that intensity range. While the d-wave implicit time was shorter than that of the b-wave in control conditions (Two-Way ANOVA, $p < 0.0001$), after treatment with PT + NMDA it became longer (Two-Way ANOVA, $p < 0.0032$). The same effect was seen during the perfusion with picrotoxin alone.

In order to reveal the contribution of each site of action (proximal and distal) to the overall PT effect on the ERG wave amplitude, we compared the amplitude changes under the influence of

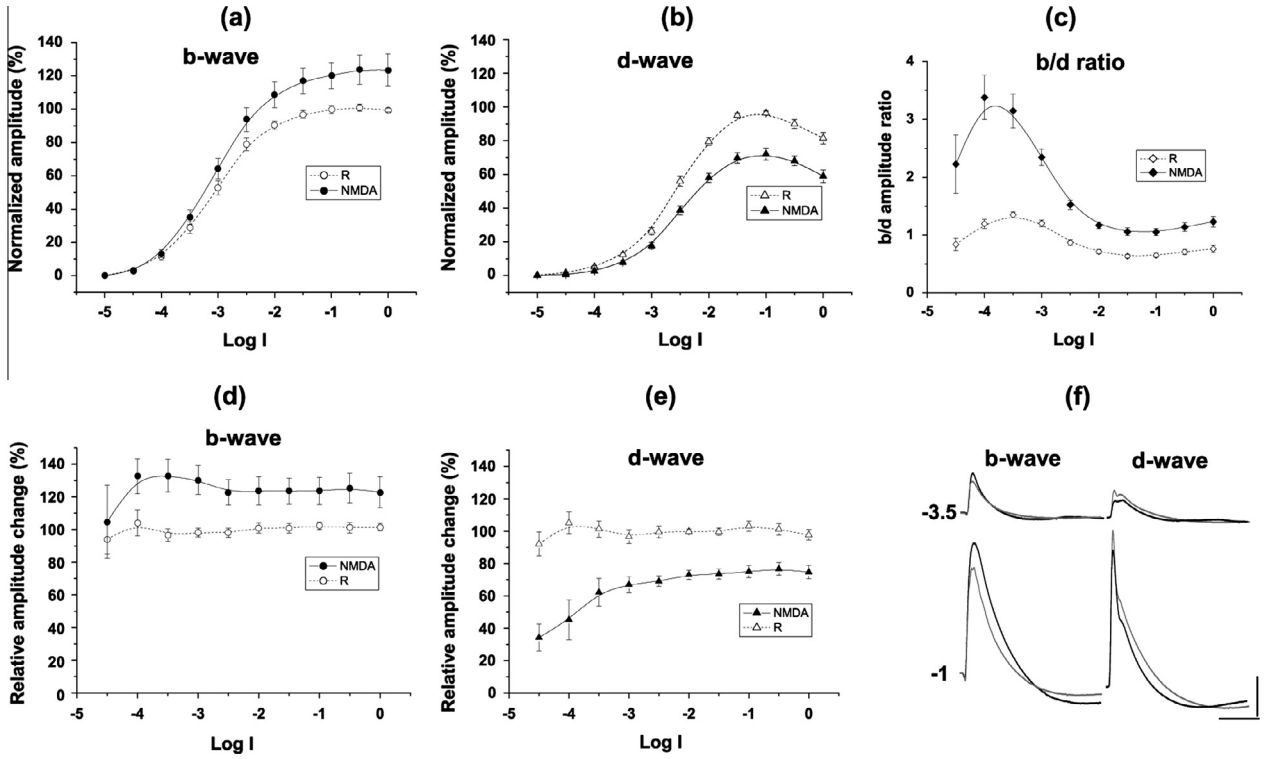

Fig. 5. (a and b) Effects of NMDA on the V–log$I$ function of the ERG b- and d-waves. The amplitudes of the ERG waves are normalized to V_{max} of the responses obtained during the first series of the experiments. The symbols representing the responses obtained during the first (open symbols) and second (filled symbols) intensity series are denoted in the legends. Mean values ± SEM are shown (n = 15). (c) Changes in the b/d amplitude ratio in the first (open symbols) and second (filled symbols) intensity series in NMDA experiments. Means ± SEM are represented. (d and e) Relative amplitude change of the ERG b-wave (d) and d-wave (e) in the control experiments (open symbols) and NMDA experiments (filled symbols). The amplitudes of the ERG waves obtained at each I_t during the second intensity series were normalized to the ERG amplitudes obtained during the first series. Mean values ± SEM are shown. (f) Original ERG records, obtained with different stimulus intensities during the control period (grey lines) and during the treatment with NMDA (black lines). The numbers on the left side indicate stimulus intensity (logI_t). Calibration: time – 500 ms, amplitude – 200 μV.

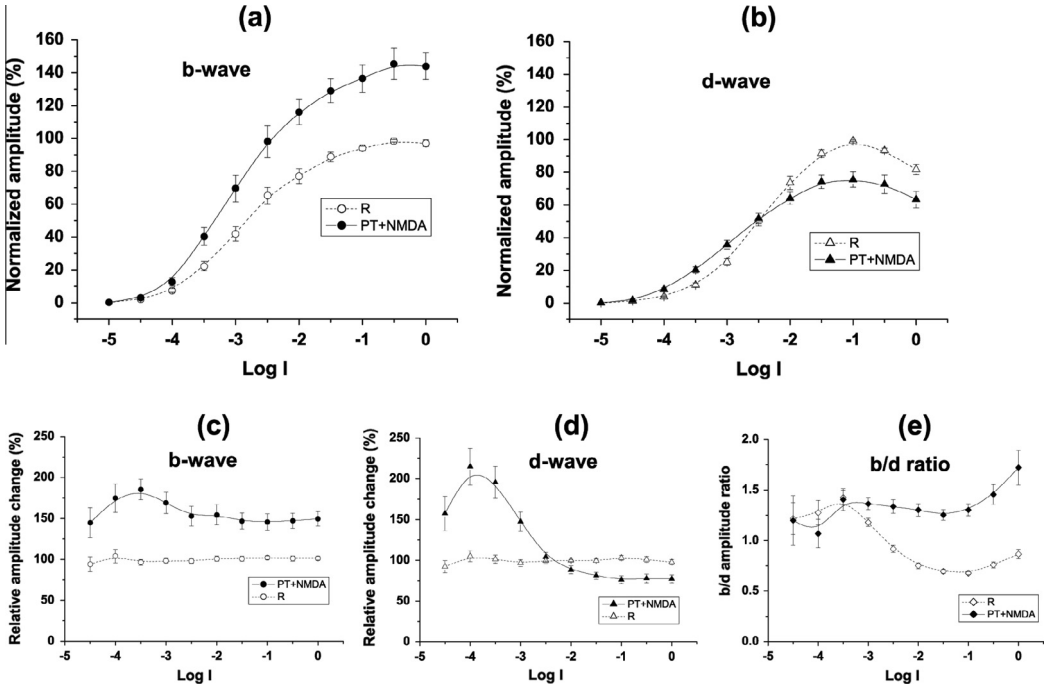

Fig. 6. (a and b) Effects of combination of picrotoxin and NMDA on the V–log$I$ function of the ERG b- and d-waves. The amplitudes of the ERG waves are normalized to V_{max} of the responses obtained during the first series of the experiments. The symbols representing the responses obtained during the first (open symbols) and second (filled symbols) intensity series are denoted in the legends. Mean values ± SEM are shown (n = 9). (c and d) Relative amplitude change of the ERG b-wave (c) and d-wave (d) in the control experiments (open symbols) and experiments with combined application of PT and NMDA (filled symbols). The amplitudes of the ERG waves obtained at each I_t during the second intensity series were normalized to the ERG amplitudes obtained during the first series. Mean values ± SEM are shown. (e) Changes in the b/d amplitude ratio in the first (open symbols) and second (filled symbols) intensity series in experiments with combined application of PT and NMDA. Means ± SEM are represented.

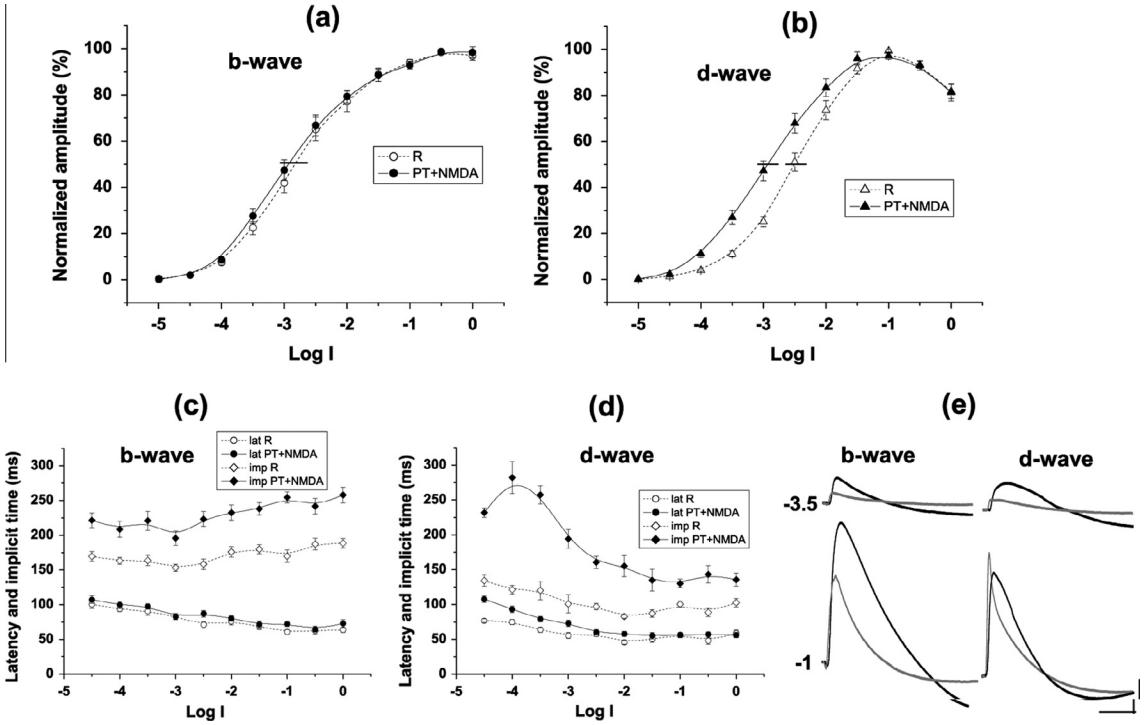

Fig. 7. (a and b) Normalized V–log$I$ curves of the b- and d-waves obtained during the first (open symbols) and the second (filled symbols) series of the experiments with combined application of PT and NMDA. The values in each series are normalized to the V_{max} obtained in the same series. This manner of representation demonstrates the changes of the position of the V–log$I$ function along the intensity axis and its steepness under the influence of PT + NMDA. The I_σ point is denoted with a black horizontal line on the curve. (c and d) Changes of the latency (lat) and implicit time (imp) in the first (open symbols) and second (filled symbols) intensity series in experiments with combined application of PT and NMDA. Means ± SEM are represented. (e) Original ERG records, obtained with different stimulus intensities during the control period (grey lines) and during the treatment with PT + NMDA (black lines). The numbers on the left side indicate stimulus intensity (logI_t). Calibration: time – 500 ms, amplitude – 200 μV.

PT + NMDA with that observed under the influence of PT and NMDA alone (Fig. 8a and b). We obtained that the b-wave amplitude during the perfusion with PT + NMDA was significantly lower than that observed during the perfusion with PT alone (Two-Way ANOVA, $p < 0.0001$), but significantly higher than that observed during the perfusion with NMDA alone over the entire intensity range (Two-Way ANOVA, $p < 0.0039$) (Fig. 8c). This result indicates that a part of the enhancing effect of PT on the b-wave amplitude was likely due to its action in the proximal retina, while another part did not depend on the activity of proximal retinal neurons. The latter part is a smaller one in the overall potentiating effect of PT on the b-wave amplitude. Its relative significance appears to be more pronounced at lower stimulus intensities compared to higher ones (Fig. 8c). When similar comparison was made for the d-wave amplitude, it was evident that its amplitude during combined application of PT and NMDA was significantly smaller in comparison to that obtained during PT treatment at all stimulus intensities (Two-Way ANOVA, $p < 0.0001$), but significantly higher than that obtained during NMDA treatment at all stimulus intensities except for the highest ones ($I_t \geqslant -1$) (Two-Way ANOVA, $p < 0.0001$). This result shows that the enhancing effect of PT on the d-wave amplitude was due to its action in both proximal and distal retina at all stimulus intensities except at the highest ones ($I_t \geqslant -1$). The proximal action has greater contribution to the overall PT effect at all stimulus intensities compared to the distal one (Fig. 8d). The distal action seems to be most important at the lowest stimulus intensities and its role gradually decreases with increasing stimulus intensity (Fig. 8d). At the highest photopic intensities only the proximal action of picrotoxin is responsible for its enhancing effect on the d-wave amplitude.

4. Discussion

Our results clearly showed that the blockade of ionotropic GABA receptors by picrotoxin enhanced the amplitude and slowed the time course of both the b- and d-waves in light adapted frog ERG over the entire intensity range studied. These results suggest that the endogenous GABA acts to decrease the amplitude and speed up the time characteristics of the cone-mediated frog ERG waves irrespective of the light intensity used. The relative strength of this action, however, depends on stimulus intensity. It is more pronounced at the lower range of stimulus intensities and thus it acts to decrease the relative sensitivity of the ERG ON and OFF responses. Similar results have been obtained by Arnarsson and Eysteinsson (1997), who have shown that picrotoxin shifts to the left the V–log$I$ function of the light adapted b- and d-waves in Xenopus ERG. Our present results suggest that the suppressing action of GABA is stronger upon the ERG OFF as compared to the ON response at the lower stimulus intensities, while the reverse is true for the higher intensity range. Thus, the b/d amplitude ratio obtained with different photopic light intensities depends in a critical manner on the GABAergic neurotransmission in frog retina. In this study we have shown also that the GABAergic system is responsible for some of the differences between the cone-mediated ON and OFF responses. This concerns their sensitivity (absolute and relative) and time characteristics. The GABAergic system is not essential, however, for the dynamic range of the b-wave V–log$I$ function, because picrotoxin changed it neither in the intact eyecups nor in the eyecups treated with NMDA. Our suggestion is not in line with the statement of Herrmann et al. (2011), who argue that one of the most important functions of GABA, acting on GABAc receptors in mouse retina, is to broaden the dynamic range of the

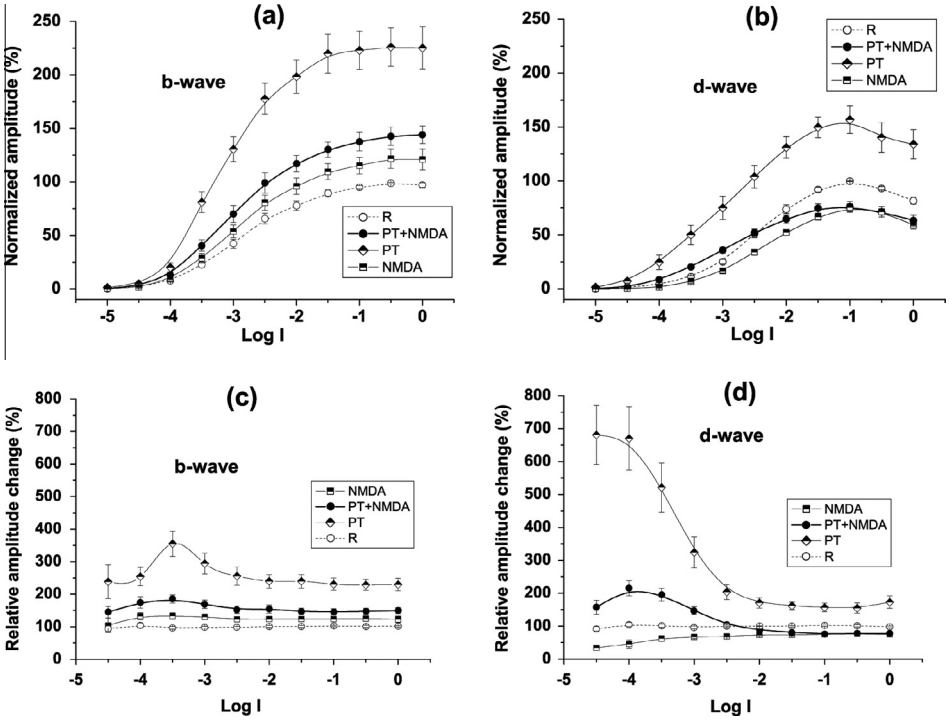

Fig. 8. (a and b) Effects of picrotoxin, NMDA and combination of picrotoxin and NMDA on the *V*–log*I* function of the ERG b- and d-waves. The amplitudes of the ERG waves are normalized to V_{max} of the responses obtained during the second series of the control experiments. Mean values ± SEM are shown. (c and d) Relative amplitude change of the ERG b-wave (c) and d-wave (d) in the control experiments (R), experiments with application of picrotoxin (PT), NMDA and combination of picrotoxin + NMDA (PT + NMDA). Mean values ± SEM are shown.

b-wave both in the dark and under background illumination. The authors cited have found that the b-wave dynamic range is narrowed in GABAc receptor knockout mice as well as in wild type mice with pharmacologically blocked GABAc receptors. However, because they did not block simultaneously both types of ionotropic GABA receptors, their results could not be directly compared with ours. The action of endogenous GABA, mediated by both $GABA_A$ and $GABA_C$ receptors, may be different from that mediated by GABAc receptors only. This suggestion is supported by the fact that Herrmann et al. (2011) did not obtain any significant effect of the exogenously applied GABA on the operational range of the b-wave responses in wild type mice, although GABA by itself increased their amplitude. All results shown in the present work are in full agreement with our previous results obtained in frog retina, where we have demonstrated that picrotoxin does not alter the dynamic range of the b-wave *V*–log*I* function, although it increases the amplitude and slows the time course of the b- and d-waves under photopic conditions of light adaptation (Kupenova, Popova, & Vitanova, 2008; Popova, 2000).

In this study we used high doses of NMDA to block the activity of proximal retinal neurons. It has been shown that NMDA depolarizes amacrine and ganglion cells and eliminates their light responses (Coleman & Miller, 1988; Dixon & Copenhagen, 1992; Lukasiewicz & McReynolds, 1985; Slaughter & Miller, 1983; Stockton & Slaughter, 1989). At the same time, NMDA has no effect on the light responses of photoreceptors and distal retinal neurons (Krizaj, Akopian, & Witkovsky, 1994; Massey & Miller, 1987; Slaughter & Miller, 1983; Stockton & Slaughter, 1989; Yang & Wu, 1991). Thus, the NMDA treatment proved to be a useful tool for assessment of proximal neuron contribution to the ERG components. In the present work we demonstrated that NMDA alone caused an increase of the b-wave amplitude and a diminution of the d-wave amplitude without altering their time course at all stimulus intensities. This indicates that the activity of proximal neurons directly contributes to generation of the cone-mediated d-wave, but not b-wave in frog ERG. The present findings confirm our previous results obtained in frog retina (Popova & Kupenova, 2009) and are consistent with the results of other authors for the amphibian b- and d-waves (Awatramani, Wang, & Slaughter, 2001; Katz et al., 1991) and mammalian b-wave (*cat*: Gargini et al., 1999; *distrophic rats*: Ohzeki et al., 2007; *rabbit*: Hare & Wheeler, 2009). There are no available data concerning the action of NMDA on the mammalian d-wave.

The present results clearly show that the pharmacological blockade of proximal retinal neurons does not eliminate the effects of picrotoxin on the ERG b- and d-waves. The enhancement of the b-wave amplitude in the eyecups treated with picrotoxin + NMDA was expressed to a greater extent than that obtained in the NMDA group at all stimulus intensities (expect for the threshold ones), indicating that it was due to picrotoxin action in the distal retina. The effects of picrotoxin on the d-wave amplitude in the NMDA treated eyecups depended on stimulus intensity. An increase of the d-wave amplitude was seen only in the lower half of the intensity range, when the amplitude values during PT + NMDA perfusion were compared with the control ones. However, when they were compared with the NMDA group, it became evident that the enhancing effect of picrotoxin upon the d-wave amplitude was preserved over the whole intensity range except for the highest stimulus intensities. Thus, we may suggest that endogenous GABA, acting in the distal frog retina, has a suppressive action on the amplitude of the cone-mediated OFF response at all stimulus intensities except for the highest ones. Our results indicate that this inhibitory action starts at very low stimulus intensities (producing d-wave amplitude $\leqslant 5$ μV) and thus significantly decreases the absolute sensitivity of the response. The GABAergic horizontal cells are the most probable source of this distal GABA action, while the GABAergic OFF amacrine cells probably account for the proximal inhibition of the ERG OFF response. Our results

suggest that the absolute sensitivity of the GABAergic horizontal cells is similar to that of the GABAergic OFF amacrine cells. On the other hand, the inhibitory action of GABA upon the ON response, exerted in distal retina, starts at higher stimulus intensities (producing b-wave amplitude between 5 μV and 10 μV) than the proximal one (I_{ts} producing b-wave amplitude $\leqslant$5 μV). Because the GABAergic ON amacrine cells are the most probable source of the proximal GABA action, one may suggest that their absolute sensitivity is higher compared to the absolute sensitivity of the GABAergic horizontal cells, which account for the distal GABA action. Such a difference in the sensitivity could be related to the higher synaptic gain in metabotropic cascade pathway of the ON bipolar cells, which feed the GABAergic ON amacrine cells, in comparison with the ionotropic glutamate transmission in the GABAergic horizontal cells (Falk & Shiells, 2006). It seems that the effects of GABA on the suprathreshold b- and d-wave amplitude, exerted in the distal and proximal retina, depend in a similar manner on stimulus intensity. Our results suggest that GABA acting in each site (distal or proximal) has stronger suppressive effect on the b- and d-wave amplitude at the lower stimulus intensities in comparison with the higher ones. Thus, its action leads to a decreased relative sensitivity of the ERG ON and OFF responses irrespective of the site it takes place. This suggestion is in line with the results of Chappell and Rosenstein (1996), who argue that picrotoxin enhancing effect upon the ON and OFF components of the skate ERG, exerted in the distal retina, is especially pronounced over a range of intensities 1 or 2 log units above threshold. The stronger effect of GABA at the lower stimulus intensities may be due in part to the fact that the gain of cone – horizontal cell synapse is highest for dim light modulation around the background illumination (Normann & Perlman, 1979). The higher gain of the cone – GABAergic horizontal cell synapse at lower stimulus intensities could generate stronger inhibitory GABAergic influences on the ON and OFF bipolar cells at these intensities.

Our present results indicate that the proximal GABA action has greater contribution to the ERG b- and d-wave amplitude shaping over a wide range of photopic intensities compared to the distal one. Our suggestion is consistent with the results of other authors working on amphibian retina, who have shown that picrotoxin greatly enhances the proximal field potentials (Karwoski, Xu, & Yu, 1996; Katz et al., 1991; Xu & Karwoski, 1994; Xu & Karwoski, 1995). However, while the authors cited above argue that picrotoxin effects on the b-and d-wave amplitude are due entirely to its action in proximal retina, our results indicate that a significant part of this action occurs in distal retina. Xu and Karwoski (1995) have showed, however, that the amplitude of the dark-evoked d-wave in the distal frog retina was also increased during the picrotoxin treatment. Although the authors did not present data concerning the statistical significance of the observed effects in distal retina, their observation is in full agreement with our present data. The discrepancy between our results and results of Katz et al. (1991) may be due to different conditions of adaptation. Katz et al. (1991) used dark adapted, isolated, toad retina preparations, stimulated by relatively low-intensity, 500 nm light stimuli, whereas we used light adapted frog eyecups. It is possible that the site of picrotoxin action in rod-mediated pathways differs from that in cone-mediated ones. However, Chappell and Rosenstein (1996), who work on all rod retina of skate, have found that NMDA does not alter picrotoxin enhancing effects on the ERG ON and OFF component and suggest that the observed effects are due to picrotoxin action in distal retina. Thus, the GABAergic rod pathways may also originate in distal retina.

Our results are in conflict with the results of Arnarsson and Eysteinsson (1997), who do not find any increase of the b-wave amplitude, caused by picrotoxin if the Xenopus retina was previously superfused with NMDA. The cause for this discrepancy is unclear, but it could not be related to different photoreceptor input, because the experiments of the both laboratories were performed on light adapted eyecups. The results presented in our study indicate also that GABA, acting in the distal retina, considerably accelerates the time course of the cone-mediated ERG waves. We showed that both the latency and implicit time of the b-and d-waves were significantly delayed by picrotoxin in eyecups, treated with NMDA. The effect was evident over the entire intensity range, indicating that the intensity of the photopic stimuli is not of critical importance for its expression. An interesting observation was that the time course of the d-wave was slowed even at the highest stimulus intensities, where its amplitude was not enhanced. Our results are consistent with the results of Karwoski, Xu, and Yu (1996), who also reported that picrotoxin increased the duration of the b- and b-waves in the distal frog retina.

The changes of the amplitude and time course of the ERG b- and d-waves under the influence of picrotoxin can be attributed mainly to changes of the light responses of the ON and OFF bipolar cells. At present, little is known about the effects of GABA and ionotropic GABA receptor antagonists on the intensity–response function of the amphibian bipolar cells. Most of the studies were performed by using one or narrow range of stimulus intensities and the results obtained are contradictory. While some authors reported that GABA diminishes and GABA antagonists (bicuculline, picrotoxin) enhance the light responses of ON bipolar cells (*mudpuppy*: Miller, Slaughter, & Dick, 1982; Miller et al., 1981), other authors have not obtained any effect of GABA on them (*xenopus*: Stone & Shutte, 1991). Still other authors suggest that the effect of GABA on the amphibian ON bipolar cells may be related only to the time characteristics of the light responses and not to their amplitude. It has been shown that SR95531 ($GABA_A$ antagonist) makes the responses slightly more transient without altering their amplitude, while picrotoxin makes them more sustained (*mudpuppy*, *tiger salamander*: Zhang, Jung, & Slaughter, 1997). Inconsistent results have been obtained also for the GABA effects on the amphibian OFF bipolar cells. Some authors reported that antagonists of ionotropic GABA receptors have no effect on their light responses (*mudpuppy*: Daniels, 1974; Miller et al., 1981), while other authors reported their suppression under the influence of GABA (Hare & Owen, 1996; Stone & Shutte, 1991) or GABA antagonists picrotoxin and bicuculline (Hare & Owen, 1996). Our results indicate that GABA, released in both the outer and inner plexiform layer in frog retina, acts to diminish the amplitude and speed up the time course of the cone-mediated light responses of both ON and OFF bipolar cells. The effect seems to be expressed over a wide range of stimulus intensities. This suggestion is not consistent with the proposed opposite action of GABA on the dendrites of ON and OFF bipolar cells in goldfish (Li & Shen, 2007) and mammalian (Vardi et al., 2000) retina. The authors cited suggest that GABA inputs in the distal retina might excite ON bipolar cells, but inhibit OFF bipolar cells, because their dendrites express different types of cation chloride cotransporters. Our data do not support such a hypothesis for the GABA action in the distal frog retina. It appears that cone-mediated light responses of frog OFF bipolar cells are more sensitive to GABA action compared to that of the ON bipolar cells, when they are obtained with low intensity light stimuli. However, the reverse statement is true for the light responses, obtained with high intensity stimuli. Our results suggest that GABA, released in the OPL, as well as GABA, released in the IPL, contributes to this ON/OFF asymmetry. Previous ERG data, obtained by us in frog retina indicate that $GABA_A$ receptors are involved to a greater extent than $GABA_C$ in establishing this ON/OFF asymmetry in the lower intensity range, while GABAc receptors contribute to it in the higher intensity range (Kupenova, Popova, & Vitanova, 2008). Our immunocytochemical study of GABA receptor distribution in frog retina has shown that $GABA_A$ receptors are located mainly in the

distal and central part of the IPL, where the OFF bipolar cell axons terminate. The greater number of these receptors on the OFF as compared to the ON bipolar cell axon terminals could account for the observed by us ON/OFF asymmetry in GABA action. It remains to be determined how GABA acting on $GABA_A$ and $GABA_C$ receptors expressed on the dendrites and axon terminals of the ON and OFF bipolar cells modulates their light responses in frog retina. It has been shown, however, that GABA elicits large $GABA_A$ and $GABA_C$ receptor mediated currents in the axon terminals, as well as in the dendrites of the both types of bipolar cells in bullfrog retina (Du & Yang, 2000). These results support our suggestion that GABA acts in both plexiform layers to modulate the light responses of the ON and OFF bipolar cells.

5. Conclusions

The results of our study indicate that GABA acts in both distal and proximal retina to modulate cone-mediated frog electroretinogram. It decreases the b- and d-wave amplitude and speed up the time course of the responses irrespective of the site of its action and the stimulus intensity used. The relative strength of this action, however, depends on stimulus intensity. It is stronger at lower than higher stimulus intensities and thus it acts to decrease the relative sensitivity of the responses. The suppressive action of GABA is expressed to a greater extent upon the OFF than ON response at the lower stimulus intensities, while the opposite is true for the higher intensity range. The obtained ON/OFF asymmetry does not depend on the site of GABA action in the retina.

References

Arnarsson, A., & Eysteinsson, T. (1997). The role of GABA in modulating the Xenopus electroretinogram. *Visual Neuroscience, 14*, 1143–1152.

Arnarsson, A., & Eysteinsson, T. (2000). Modification of the Xenopus electroretinogram by action of glycine in the proximal retina. *Acta Physiologica Scandinavica, 169*, 249–258.

Awatramani, G., Wang, J., & Slaughter, M. M. (2001). Amacrine and ganglion cell contributions to the electroretinogram in amphibian retina. *Visual Neuroscience, 18*, 147–156.

Belcheva, S., & Kupenova, P. (1980). Effects of picrotoxin and strychnine on the frog electroretinogram at different stimulus intensities. *Acta Physiologica et Pharmacologica Bulgarica, 6*, 14–15.

Belcheva, S., & Vitanova, L. (1974). Effects of some antagonists of the inhibitory transmitters of the bioelectrical activity of the retinal cells. *Agressologie, 15*, 461–469.

Belcheva, S., & Vitanova, L. (1978). Effects of picrotoxin and strychnine on the bioelectric activity of the turtle's retinal neurons. *Agressologie, 19*, 309–320.

Bonaventure, N., Wioland, N., & Jardon, B. (1986). Anisotropic inhibition in the receptive field surround of the frog retinal ganglion cells, evidenced by bicuculline and SR 95103, a new GABA antagonist. *European Journal of Pharmacology, 121*, 327–336.

Chappell, R. L., & Rosenstein, F. J. (1996). Pharmacology of the skate electroretinogram indicates independent ON and OFF bipolar cell pathways. *Journal of General Physiology, 107*, 535–544.

Chappell, R. L., Schuette, E., Anton, R., & Ripps, H. (2002). GABAc receptors modulate the rod-driven ERG b-wave of the skate retina. *Documenta Ophthalmologica, 105*, 179–188.

Coleman, P. A., & Miller, R. F. (1988). Do N-methyl-D-aspartate receptors mediate synaptic responses in the mudpuppy retina. *Journal of Neuroscience, 8*, 4728–4733.

Connaughton, V. P., Nelson, R., & Bender, A. M. (2008). Electrophysiological evidence of $GABA_A$ and $GABA_C$ receptors on zebrafish retinal bipolar cells. *Visual Neuroscience, 25*, 139–153.

Daniels, J. D. (1974). *Synaptic transmission in the vertebrate retina: Pharmacology via intracellular recordings*. Ph.D. thesis, University of California, Berkeley, California, USA.

DeVries, G. W., & Friedman, A. H. (1978). GABA, picrotoxin and retinal sensitivity. *Brain Research, 148*, 530–535.

Dick, E., & Miller, R. F. (1978). Light-evoked potassium activity in mudpuppy retina: Its relationship to the b-wave of the electroretinogram. *Brain Research, 154*, 388–394.

Dick, E., & Miller, R. F. (1985). Extracellular K^+-activity changes related to electroretinogram components I: Amphibian (l-type) retinas. *Journal of General Physiology, 85*, 885–909.

Dick, E., Miller, R. F., & Daucheux, R. F. (1979). Neuronal origin of the b- and d-waves in the I-type ERG. *Investigative Ophthalmology and Visual Science, 18*(Suppl.), 34.

Dixon, D. B., & Copenhagen, D. R. (1992). Two types of glutamate receptors differentially excite amactine cells in the tiger salamander retina. *Journal of Physiology, 449*, 589–606.

Dong, C. J., & Hare, W. A. (2002). GABAc feedback pathway modulates the amplitude and kinetics of ERG b-wave in a mammalian retina in vivo. *Vision Research, 42*, 1081–1087.

Du, J. L., & Yang, X. L. (2000). Subcellular localization and complements of GABA(A) and GABA(C) receptors on bullfrog retinal bipolar cells. *Journal of Neurophysiology, 84*, 666–676.

Eggers, E. D., Ichinose, T., Sagdullaev, B. T., & Lukasiewicz, P. D. (2006). Retinal GABA receptors and visual processing: A model system for presynaptic inhibition. *Cellscience Reviews, 2*, 50–67.

Eggers, E. D., & Lukasiewicz, P. D. (2011). Multiple pathways of inhibition shape bipolar cell responses in the retina. *Visual Neuroscience, 28*, 95–108.

Enz, R., Brandstätter, J. H., Wässle, H., & Bormann, J. (1996). Immunocytochemical localization of the GABAC receptor r subunits in the mammalian retina. *Journal of Neuroscience, 16*, 4479–4490.

Euler, T., & Wässle, H. (1998). Different contributions of GABAA and GABAC receptors to rod and cone bipolar cells in a rat retinal slice preparation. *Journal of Neurophysiology, 79*, 1384–1395.

Fain, G. L. (1976). Sensitivity of toad rods: Dependence on wave-length and background illumination. *Journal of Physiology, 261*, 71–101.

Falk, G., & Shiells, R. (2006). Synaptic transmission: Sensitivity control mechanisms. In J. R. Heckenlively & G. B. Arden (Eds.), *Principles and practice of clinical electrophysiology of vision* (pp. 79–91). Cambridge, Massachusetts, London, England: The MIT Press.

Fletcher, E. L., Koulen, P., & Wässle, H. (1998). GABAA and GABAC receptors on mammalian rod bipolar cells. *Journal of Comparative Neurology, 396*, 351–365.

Frishman, L. J., & Steinberg, R. H. (1990). Origin of negative potentials in the light-adapted ERG of cat retina. *Journal of Neurophysiology, 63*, 1333–1346.

Gargini, C., Demontis, G. C., Cervetto, L., & Bisti, S. (1999). Analysis of pharmacologically isolated components of the ERG. *Vision Research, 39*, 1759–1766.

Gottlob, I., Wündsch, L., & Tuppy, F. K. (1988). The rabbit electroretinogram: Effect of GABA and its antagonists. *Vision Research, 28*, 203–210.

Green, D. G., & Kapousta-Bruneau, N. V. (1999). A dissection of the electroretinogram from the isolated rat retina with microelectrodes and drugs. *Visual Neuroscience, 16*, 727–741.

Grünert, U. (2000). Distribution of GABA and glycine receptors on bipolar and ganglion cells in the mammalian retina. *Microscopy Research and Technique, 50*, 130–140.

Gurevich, L., & Slaughter, M. M. (1993). Comparison of the waveforms of the ON bipolar neuron and the b-wave of the electroretinogram. *Vision Research, 33*, 2431–2435.

Hanitzsch, R., Lichtenberger, T., & Mattig, W. U. (1996). The influence of $MgCl_2$ and APB on the light-induced potassium changes and the ERG b-wave of the isolated superfused rat retina. *Vision Research, 6*, 499–507.

Hare, W. A., & Owen, W. G. (1996). Receptive field of the retinal bipolar cell: A pharmacological study in the tiger salamander retina. *Journal of Neurophysiology, 76*, 2005–2019.

Hare, W. A., & Wheeler, L. (2009). Experimental glutamatergic excitotoxicity in rabbit retinal ganglion cells: Block by memantidine. *Investigative Ophthalmology and Visual Science, 50*, 2940–2948.

Herrmann, R., Heflin, S. J., Hammond, T., Lee, B., Wang, J., Gainetdinov, R. R., et al. (2011). Rod vision is controlled by dopamine-dependent sensitization of rod bipolar cells by GABA. *Neuron, 72*, 101–110.

Hood, D. C., & Hock, P. A. (1975). Light adaptation of the receptors: Increment threshold function for the frog's rods and cones. *Vision Research, 15*, 545–553.

Kalloniatis, M., & Tomisich, G. (1999). Amino acid neurochemistry of the vertebrate retina. *Progress in Retinal and Eye Research, 18*, 811–866.

Kaneko, A., & Tachibana, M. (1986). Effects of γ-aminobutyric acid on isolated cone photoreceptors of the turtle retina. *Journal of Physiology (London), 373*, 443–461.

Kapousta-Bruneau, N. V. (2000). Opposite effects of GABA(A) and GABA(C) receptor antagonists on the b-wave of ERG recorded from the isolated rat retina. *Vision Research, 40*, 1653–1665.

Karwoski, C. J., & Xu, X. (1999). Current source-density analysis of light-evoked field potentials in rabbit retina. *Visual Neuroscience, 16*, 369–377.

Karwoski, C. J., Xu, X., & Yu, H. (1996). Current source-density analysis of the electroretinogram of the frog: Methodological issues and origin of components. *Journal of the Optical Society of America A: Optics, Image Science, and Vision, 13*, 549–556.

Katz, B. J., Wen, R., Zheng, J., Xu, Z., & Oakley, I. I. (1991). M-wave of the toad electroretinogram. *Journal of Neurophysiology, 66*, 1927–1940.

Koulen, P., Brandstätter, J. H., Enz, R., Bormann, J., & Wässle, H. (1998). Synaptic clustering of GABAC receptor r-subunits in the rat retina. *European Journal of Neuroscience, 10*, 115–127.

Krizaj, D., Akopian, A., & Witkovsky, P. (1994). The effects of L-glutamate, AMPA, quisqualate and kainite on retinal horizontal cells depend on adaptational state: Implications for rod–cone interactions. *Journal of Neuroscience, 14*, 5661–5671.

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

Kupenova, P., Popova, E., & Vitanova, L. (2008). GABAa and GABAc receptor mediated influences on the intensity–response functions of the b- and d-wave in the frog ERG. *Vision Research, 48*, 882–892.

Kupenova, P., Vitanova, L., Mitova, L., & Belcheva, S. (1991). Participation of the GABAergic system of the turtle retina in the light adaptation process. *Acta Physiologica Scandinavica, 143*, 203–210.
Kupenova, P., Vitanova, L., Popova, E., & Mitova, L. (1997). Influence of picrotoxin and strychnine on the spectral sensitivity of the turtle ERG b- and d-wave: I. Dark adaptation. *Acta Physiologica Scandinavica, 159*, 217–225.
Lewis, A., Wilson, N., Stearns, G., Johnson, N., Nelson, R., & Brockerhoff, S. E. (2011). Celsr3 is required for normal development of GABA circuits in the inner retina. *PLoS Genetics, 7*, e1002239.
Li, B., & Shen, W. (2007). Cation Cl^- cotransporters in the dendrites of goldfish bipolar cells. *NeuroReport, 18*, 625–629.
Lin, Z. S., & Yazulla, S. (1994). Heterogeneity of GABAA receptor in goldfish retina. *Journal of Comparative Neurology, 345*, 429–439.
Lukasiewicz, P. D., Eggers, E. D., Sagdullaev, B. T., & McCall, M. A. (2004). $GABA_C$ receptor-mediated inhibition in the retina. *Vision Research, 44*, 3289–3296.
Lukasiewicz, P. D., & McReynolds, J. S. (1985). Synaptic transmission at N-methyl-D-aspartate receptors in the proximal retina of the mudpuppy. *Journal of Physiology, 367*, 99–115.
Lukasiewicz, P. D., & Shields, C. R. (1998). A diversity of GABA receptors in the retina. *Seminars in Cell and Developmental Biology, 9*, 293–299.
Lukasiewicz, P. D., & Wong, R. O. L. (1997). GABAC receptors on ferret bipolar cells: A diversity of subtypes in mammals? *Visual Neuroscience, 14*, 989–994.
Massey, S. C., & Miller, R. F. (1987). Excitatory amino acid receptors of rod- and cone-driven horizontal cells in the rabbit retina. *Journal of Neurophysiology, 63*, 16–30.
Miller, R. F., Frumkes, T. E., Slaughter, M. M., & Dacheux, R. F. (1981). Physiological and pharmacological basis of GABA and glycine action on neurons of mudpuppy retina. I. Receptors, horizontal cells, bipolars and G-cells. *Journal of Neurophysiology, 45*, 743–763.
Miller, R. F., Slaughter, M. M., & Dick, E. (1982). Excitatory, inhibitory and peptidergic pathways in the mudpuppy retina. In Bradford (Ed.), *Neurotransmitter interaction and compartmentation* (pp. 735–759). New York: Plenum Press.
Naarendorp, F., & Sieving, P. A. (1991). The scotopic threshold response of the cat ERG is suppresses selectively by GABA and glycine. *Vision Research, 31*, 1–15.
Naka, K. I., & Rushton, W. A. H. (1966). S-potentials from colour units in the retina of fish (Cyprinidae). *Journal of Physiology, 185*, 536–555.
Newman, E. A., & Odette, L. L. (1984). Model of electroretinogram b-wave generation: A test of the K^+ hypothesis. *Journal of Neurophysiology, 51*, 164–182.
Normann, R. A., & Perlman, I. (1979). Signal transmission from red cones to horizontal cells in the turtle retina. *Journal of Physiology, 286*, 509–524.
Ohzeki, T., Machida, S., Takahashi, T., Ohtaka, K., & Kurosaka, D. (2007). The effect of intravitreal N-methyl-DL-aspartic acid on the electroretinogram in Royal College of surgeons rats. *Japanese Journal of Ophthalmology, 51*, 165–174.
Pattnaik, B., Jellali, A., Sahel, J., Dreyfus, H., & Picaud, S. (2000). GABAC receptors are localized with microtubule-associated protein 1B in mammalian cone photoreceptors. *Journal of Neuroscience, 20*, 6789–6796.
Picaud, S., Pattnaik, B., Hicks, D., Forster, V., Fontaine, V., Sahel, J., et al. (1998). $GABA_A$ and $GABA_C$ receptors in adult porcine cones: Evidence from a photoreceptor-glia co-culture model. *Journal of Physiology (London), 513*, 33–42.
Popova, E. (1989). Picrotoxin effects on frog ERG at different background illumination but same stimulus contrast. *Physiologia Bohemoslovaca, 38*, 327–337.
Popova, E. (2000). Glycinergic and GABAergic control of intensity–response function of frog ERG waves under different conditions of light stimulation. *Acta Physiologica Scandinavica, 170*, 225–242.
Popova, E., Belcheva, S., Tzekov, R., & Penchev, A. (1986). Picrotoxin effects on the frog electroretinogram under different background illumination. *Acta Physiologica et Pharmacologica Bulgarica, 12*, 32–41.
Popova, E., & Kupenova, P. (2009). Contribution of proximal retinal neurons to b- and d-wave of frog electroretinogram under different conditions of light adaptation. *Vision Research, 49*, 2001–2010.
Popova, E., & Kupenova, P. (2011). Effects of dopamine D_1 receptor blockade on the intensity–response function of ERG b- and d-waves under different conditions of light adaptation. *Vision Research, 51*, 1627–1636.
Robson, J. G., & Frishman, L. J. (1995). Response linearity and kinetics of the cat retina: The bipolar cell component of the dark-adapted electroretinogram. *Visual Neuroscience, 12*, 837–850.
Shields, C. R., Tran, M. N., Wong, R. O., & Lukasiewicz, P. D. (2000). Distinct ionotropic GABA receptors mediate presynaptic and postsynaptic inhibition in retinal bipolar cells. *Journal of Neuroscience, 20*, 2673–2682.
Shiells, R. A., & Falk, G. (1999). Contribution of rod, on-bipolar, and horizontal cell light responses to the ERG of dogfish retina. *Visual Neuroscience, 16*, 503–511.
Sieving, P. A., Murayama, K., & Naarendorp, F. (1994). Push–pull model of the primate photopic electroretinogram: A role for hyperpolarizing neurons in shaping the b-wave. *Visual Neuroscience, 11*, 519–532.
Slaughter, M. M., & Miller, R. F. (1983). The role of excitatory amino acid transmitters in the mudpuppy retina: An analysis with kainic acid and N-methyl aspartate. *Journal of Neuroscience, 3*, 1701–1711.
Starr, M. S. (1975). The effects of various amino acids, dopamine and some convulsants on the electroretinogram of the rabbit. *Experimental Eye Research, 21*, 79–87.
Stockton, R. A., & Slaughter, M. M. (1989). B-wave of the electroretinogram: A reflection of on-bipolar cell activity. *The Journal of General Physiology, 93*, 101–122.
Stone, S., & Shutte, M. (1991). Physiological and morphological properties of OFF- and ON-center bipolar cells in the Xenopus retina: Effects of glycine and GABA. *Visual Neuroscience, 7*, 363–376.
Tian, N., & Slaughter, M. M. (1995). Correlation of dynamic responses on the ON bipolar neuron and the b-wave of electroretinogram. *Vision Research, 35*, 1359–1364.
Ueno, S., Kondo, M., Ueno, M., Miyata, K., Terasaki, H., & Miyake, Y. (2006). Contribution of retinal neurons to d-wave of primate photopic electroretinogram. *Vision Research, 46*, 658–664.
Vardi, N., Masarachia, P., & Sterling, P. (1992). Immunoreactivity to GABAA receptor in the outer plexiform layer of the cat retina. *Journal of Comparative Neurology, 320*, 394–397.
Vardi, N., Morigiwa, K., Wang, T. L., Shi, Y. J., & Sterling, P. (1998). Neurochemistry of the mammalian cone 'synaptic complex'. *Vision Research, 38*, 1359–1369.
Vardi, N., Zhang, L.-L., Payne, J. A., & Sterling, P. (2000). Evidence that different cation chloride cotransporters in retinal neurons allow opposite responses to GABA. *Journal of Neuroscience, 20*, 7657–7663.
Vigh, J., & von Gersdorff, H. (2005). Prolonged reciprocal signaling via NMDA and GABA receptors at a retinal ribbon synapse. *Journal of Neuroscience, 2*, 11412–11423.
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 Research, 41*, 691–704.
Vitanova, L., Kupenova, P., Popova, E., & Mitova, L. (1997). Influence of picrotoxin and strychnine on the spectral sensitivity of the turtle ERG b- and d- wave: II. Light adaptation. *Acta Physiologica Scandinavica, 159*, 227–235.
Wachtmeister, L. (1980). Further studies of the chemical sensitivity of the oscillatory potentials of the electroretinogram (ERG) I. GABA- and glycine antagonists. *Acta Ophthalmologica (Copenhagen), 58*, 712–725.
Wässle, H., Koulen, P., Brandstätter, J. H., Fletcher, E. L., & Becker, C. M. (1998). Glycine and GABA receptors in the mammalian retina. *Vision Research, 38*, 1411–1430.
Wu, S. M., & Maple, B. R. (1998). Amino acid neurotransmitters in the retina: A functional overview. *Vision Research, 38*, 1371–1384.
Xu, X., & Karwoski, C. J. (1994). Current source density analysis of retinal field potentials. I. Pharmacological analysis of the b-wave and M-wave. *Journal of Neurophysiology, 72*, 96–105.
Xu, X., & Karwoski, C. J. (1995). Current source density analysis of the electroretinographic d-wave of frog retina. *Journal of Neurophysiology, 73*, 2459–2469.
Yanagida, T., Koshimizu, M., Kawasaki, K., & Yonemura, D. (1986). Microelectrode depth study of electrographic b- and d-waves in frog retina. *Japanese Journal of Ophthalmology, 30*, 298–305.
Yang, X.-L. (2004). Characterization of receptors fpr glutamate and GABA in retinal neurons. *Progress in Neurobiology, 73*, 127–150.
Yang, C. Y., Lin, Z. S., & Yazulla, S. (1992). Localization of GABAA receptor subtypes in the tiger salamander retina. *Visual Neuroscience, 8*, 57–64.
Yang, X.-L., & Wu, S. M. (1991). Coexistence and function of glutamate receptor subtypes in the horizontal cells of the tiger salamander retina. *Visual Neuroscience, 7*, 377–382.
Yazulla, S. (1986). GABAergic mechanisms in the retina. In J. Chader & N. Osborne (Eds.), *Progress in retinal research* (pp. 1–52). New York: Pergamon Press.
Yazulla, S., & Studholme, K. M. (1997). Light adaptation affects synaptic vesicle density but not the distribution of GABAA receptors in goldfish photoreceptor terminals. *Microscopy Research and Technique, 36*, 43–56.
Zhang, J., Jung, C. S., & Slaughter, M. (1997). Serial inhibitory synapses in retina. *Visual Neuroscience, 14*, 553–563.