



Effects of the intravitreal administration of dopaminergic ligands on the b-wave amplitude of the rabbit electroretinogram

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Abstract

In the retina of mammals, dopamine (DA) is generally released by amacrine cells and is known to alter the physiology of most retinal cells. It is well known that DA reduces the amplitude of the b-wave of the electroretinogram (ERG) in rabbit. However, the specific receptor subtypes that mediate this action have not yet been elucidated. To do this, we recorded flash ERGs before and after the intravitreal injection of D₁-like DA receptor agonists (SKF38393, A77693) and antagonist (SCH23390), and of D₂-like agonist (R(-)-propylnorapomorphine hydrochloride; NPA) and antagonist ((S)-(-)-sulpiride). Contralateral control eyes were injected with the vehicle only. Both D₁ agonists provoked a reduction of the ERG b-wave amplitude (34.0% and 59.2% of the pre-injection level, respectively). The D₂-like agonist NPA had no significant effects on ERG components. Unexpectedly, both D₁- and D₂-like antagonists also reduced the b-wave amplitude (28.9% and 59.8%). Overall, these data suggest that the previously described effect of DA on the rabbit ERG b-wave came from activation of D₁-like receptors. On the basis of the effects observed with D₂-like antagonist, a subtle contribution of D₂-like presynaptic receptors cannot be ruled out.

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1. Introduction

The retina of all mammals contains dopamine (DA). In most cases, including rabbits, this catecholamine is released by a sub-type of amacrine cells (Dowling & Ehinger, 1978) which are generally located in the proximal part of the inner nuclear layer and ramify extensively in one or more levels of the inner plexiform layer (Mitrofanis, Vigny, & Stone, 1988). Five subtypes of dopaminergic receptors have been described and designated D₁ through D₅. On the basis of their structure,

binding properties, and intracellular action, these receptors can be grouped into two subfamilies: (1) the D₁-like receptors (D₁ and D₅) which, when activated, stimulate adenylyl cyclase activity and cAMP formation; (2) the D₂-like receptors (D₂, D₃, and D₄) which inhibit adenylyl cyclase. The activity of all neurons in the retina can be modulated by DA because they all exhibit D₁-, D₂-like or both receptor subfamilies. A number of studies have revealed the implication of DA in various forms of retinal processing such as light adaptation (Marshak, 2001) and horizontal cell coupling (He, Weiler, & Vaney, 2000). Because of the latter action, DA is believed to modulate the center-surround antagonism of retinal receptive fields (Bodis-Wollner, 1996; Bodis-Wollner & Tzelepi, 1998; Boumghar, Marois, Jolicoeur, & Casanova, 1997).

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In rabbit, it has been shown that the intravitreal administration of DA (Gottvall & Textorius, 2003; Textorius, Nilsson, & Andersson, 1989) yields a reduction in the amplitude of the b-wave of the flash electroretinogram (ERG) (Jagadeesh & Sanchez, 1981). Comparable results were obtained after the i.v. injection of apomorphine, which preferentially binds to D₂-like receptors (Gao et al., 1990). Our laboratory showed that the acute administration of apomorphine could induce a change in the contrast sensitivity of neurons in the lateral geniculate nucleus, i.e., the main thalamic target of ganglion cells (Boumghar et al., 1997). Conversely, the destruction of the retinal DAergic network by the neurotoxin 6-hydroxydopamine (6-OHDA), was shown to increase the amplitude of the ERG b-wave (Lafond et al., 1994; Olivier, Jolicoeur, Lafond, Drumheller, & Brunette, 1986, 1987).

Despite the bulk of neuroanatomical and physiological evidence showing that DA can modulate the activity of retinal cells in mammals, very little is known about the specific contribution of DA receptor subtypes in retinal functioning. While the observation that apomorphine mimics the effects of DA on the ERG suggests that D₂-like receptors may subtend the retinal physiological impact of DA, the contribution of D₁-like receptors cannot be ruled out because they present, depending of the enantiomer, substantial affinity to apomorphine (Baldessarini, Kula, Zong, & Neumeyer, 1994; De Keyser, De Backer, Wilczak, & Herroelen, 1995). In the present study, we further investigated in vivo the contribution of D₁- and D₂-like receptors in the established DA-mediated reduction of the b-wave amplitude in rabbits. Parts of these findings have been presented in abstract form (Huppé-Gourgues, Coudé, & Casanova, 2002).

2. Methods

2.1. Animal preparation

Experiments were carried out on 42 adult pigmented rabbits (2.5–3.5 kg) that were provided by the Université de Montréal animal facilities. Animals were treated according to the guidelines of the Canadian Council on Animal Care. A 12 h light/dark cycle was used to maintain the animals in a normal day–night cycle. All procedures described below started in the morning to minimize any influence of the circadian cycle. Atravet (0.5 mgkg⁻¹) and atropine (0.1 mgkg⁻¹) were administered to the animal 30 minutes before general anesthesia that was induced by urethane (1.5 gkg⁻¹ i.v.) (Sigma–Aldrich), administered via the marginal vein of the ear. All surgical wounds and pressure points were infused with a local anesthetic (Lidocaine hydrochloride 2%). Heart rate and O₂ blood saturation were constantly

monitored with an oxymeter (Nonin). A tracheotomy was performed. Deep tendon reflexes were checked to ensure a satisfactory level of anesthesia during the surgery. The animal was then placed in a stereotaxic frame and muscular relaxation was obtained by injecting gallamine triethiodide (10 mgkg⁻¹h⁻¹, i.v.) mixed in dextrose ringer (5%). The animal was then artificially ventilated (N₂O/O₂: 70/30% plus halothane 0.5–1%). End-tidal CO₂ level was maintained between 28 and 32 mmHg by adjusting the stroke volume and respiratory rate. The electrocardiogram was continuously monitored, and the core temperature was maintained at 37–38 °C by a feedback-controlled heating pad placed under the animal. Pupils were dilated with isopto-atropine (1%) and nictitating membranes were retracted with local application of phenylephrine hydrochloride (2.5%). The corneas were protected from desiccation by applying artificial tears (carboxyl methylcellulose).

2.2. Recordings

ERG-Jet contact lens electrodes (Universo S.A., Switzerland) were placed on the cornea of the two eyes. Two subdermal electrodes were positioned on the bridge of the nose and between the ears on the top of the skull and served as ground and reference, respectively. Given that DAergic substances can have an effect on pupil dilation (e.g. Corbett, Buckley, & Richards, 1994) and that the testing period lasted for several hours, artificial pupils of 6 mm were used in some experiments. In these cases, the ERG was recorded with a DTL fiber (Hébert, Vaegan, & Lachapelle, 1999). No differences were noted between the effects of a given ligand on the ERG whether artificial pupils or regular jet electrodes were used. Moreover, casual observation of the pupil did not reveal any significant changes of its diameter during the testing periods.

Signals were amplified 5000× (P511, Grass, West Warwick, RI) and band-pass filtered between 1 and 1000 Hz. Signals were then fed to an analogue-digital interface (1401plus, CED, Cambridge, UK) and were acquired using the software Signal (v.2.0x, CED, Cambridge UK). A custom loop program allowed us to record ERGs at exactly 15 min apart for the whole duration of the experimental procedure. To provide a more uniform stimulation (Ganzfeld-like stimulation) of the retina, ping-pong balls cut in half covered the eyes.

2.3. Visual stimulation

Visual stimulation consisted of a series of stroboscopic flashes (single flash duration of 10 μs) of different frequencies and intensities generated by a photic stimulator PS33plus (Grass Instruments Co., West Warwick, RI). The flash lamp was placed 45 cm above the animal's head to allow for the simultaneous stimulation of the

two eyes. For each animal, the effects of the DA ligands on the ERG were studied in the two luminance conditions defined in the ISECV standards (Marmor & Zrenner, 1998), and in a third intermediate condition. In scotopic conditions, flashes were presented at a frequency (f) of 0.1 Hz, and at an intensity (i) of 0.1 cdm^{-2}s , the background luminance (bl) being at 0 cdm^{-2} . In this condition, each ERG waveform represents an average of five responses. In photopic conditions ($i = 3 \text{ cdm}^{-2}\text{s}$, $bl = 30 \text{ cdm}^{-2}$), flashes of 1 Hz were used, and each ERG consisted of an average of 30 responses. In the intermediate condition in which both rods and cones contribute to vision (namely mesopic), parameters were set at: $f = 0.5 \text{ Hz}$, $i = 2 \text{ cdm}^{-2}\text{s}$ and $bl = 13 \text{ cdm}^{-2}$. Background illumination corresponded to the room illumination by tungsten lamps (3200 K). Responses were averaged 15 times. All luminance values were obtained at the level of the animal's eye with a photometer.

2.4. Dopaminergic agents

The active substances used to characterize the contribution of D_1 -like receptor subtypes were the agonists (\pm)-SKF-38393 hydrochloride [(\pm) -1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol hydrochloride; Sigma] and A-77636 hydrochloride [$(-)$ -(1R,3S)-3-adamantyl-1-(aminomethyl)-3,4-dihydro-5,6-dihydroxy-1H-2-benzopyran hydrochloride; RBI-Sigma], and the antagonist R(+)-SCH-23390 hydrochloride [R(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride]. The involvement of D_2 receptors was assessed by administering the agonist R(-)-propylnorapomorphine hydrochloride [R(-)-10,11-dihydroxy-*N-n*-propylnorapomorphine hydrochloride; (NPA) Sigma], and the antagonist, (S)-(-)-sulpiride, [(S)-5-aminosulfonyl-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-2-methoxybenzamide; Sigma]. For clarity, the terms SKF-38393, A-77636, SCH-23390, NPA, and sulpiride will be used throughout. The ligands were dissolved in sterile water, with the exception of sulpiride which was dissolved in 0.1 N HCl. Concentrations used varied from 1 to 0.00001 mg in the injection volume of 100 μl . All solutions had a pH of ~ 7 , with the exception of sulpiride-HCl and HCl-vehicle solutions for which the pH was ~ 1.5 in both cases. Injections were made in the vitreous humor i.e., a volume of $\sim 1.4 \text{ ml}$; (Green, Leopold, & Sawyer, 1957) by inserting a 30G needle (Becton Dickinson and Co, Franklin Lakes, NJ) attached to a 250 μl Hamilton syringe throughout the sclera, 2–3 mm caudal to the limbus.

2.5. Experimental protocol

After a 1 h period of adaptation to the lightning conditions, binocular ERGs were recorded every 15 min for

a control period of 2 h. This was done to determine baseline amplitude of the ERG components of the two eyes (normalized response). The DAergic agent was then injected intravitreally into the test eye (T0), and vehicle (i.e., sterile water in all cases, except for Sulpiride experiments, in which the HCl solution was used) was injected into the control eye. Recordings were then made every 15 min after the injection. Flashes were interrupted between each recording session, and the animal was kept in the background illumination condition used in the given protocol. In general, recordings lasted 5 h after the drug administration, but could be prolonged up to 24 h. In general, after recovery, a second injection protocol was initiated with the same animal, in identical lighting conditions. The former control eye was then used as the test eye, and vice-versa, and the same drug was injected. Therefore the n values provided throughout represent the actual number of injections.

Once the last recording was made, the animal was killed with an i.v. injection of about 1.5 ml of pentobarbital sodium (Euthanyl 110 mg kg^{-1}). Eyeballs were removed and dissected for a gross evaluation of any trauma resulting from the injection procedure, and particularly to verify that no structure (e.g. lens) was inadvertently nicked by the needle.

We measured time-to-peak and amplitude just before, and at predetermined time intervals after, the injection of test agents. Post-injection b-wave amplitudes were expressed as percent of pre-injection amplitudes. In addition, the amplitude computed at each hour post-injection was statistically compared to that recorded from the control eye. The b-wave of the latter was never significantly modified throughout the recording session. Statistical significance ($p \leq 0.05$) was determined with paired *t*-test, oneway ANOVA for parametric data and Kruskal–Wallis and Wilcoxon test for non-parametric data.

3. Results

3.1. D_1 -like receptor contribution to the b-wave amplitude

The intravitreal injection of the D_1 -like agonist SKF38393 reduced the amplitude of the ERG signal. A representative example is shown in Fig. 1. SKF38393 reduced the b-wave amplitude by 30%, while the latency of the waveform remained constant at $82.3 \pm 0.3 \text{ ms}$ (panel A, upper traces). The vehicle alone did not modify the ERG of the control eye (bottom traces). Panel B represents the time course of the action of the agonist. The maximal SKF38393-induced reduction was observed between 1 and 2 h post-injection. A recovery was apparent 4 h after injection and was virtually completed after 7 h (see also the upper-right ERG waveform in panel A). Panel B also shows the

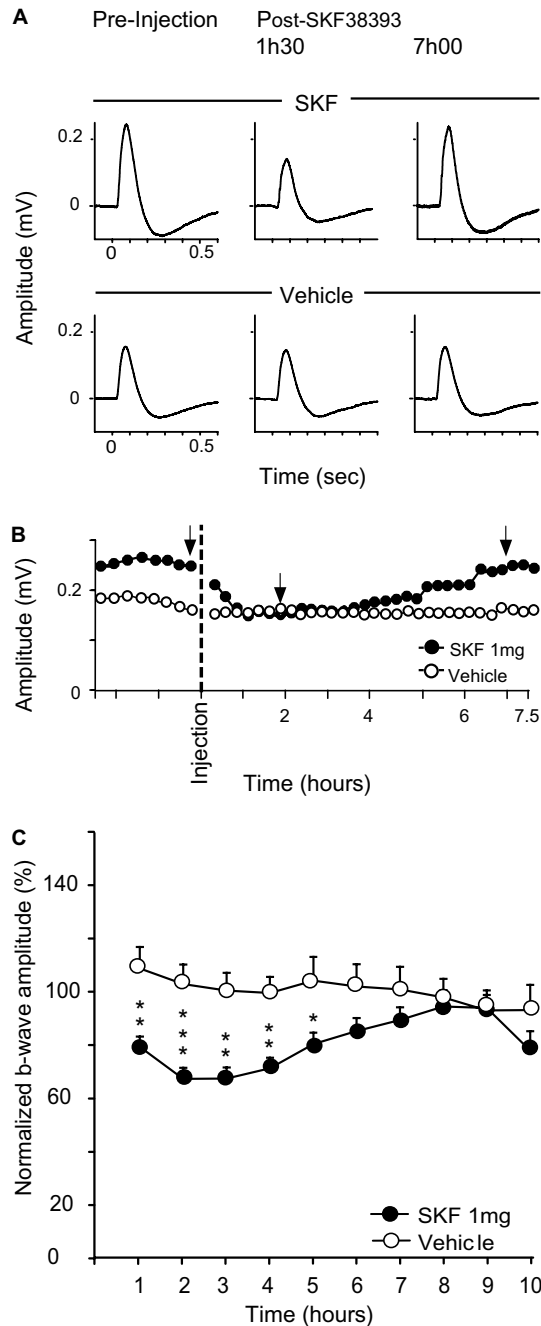


Fig. 1. (A) Representative example of ERGs before and after the intravitreal injection of D_1 -like receptors agonist, SKF38393 at $1 \text{ mg}(100 \mu\text{l})^{-1}$ (top traces) in scotopic condition. There is a reduction of about 30% of the b-wave amplitude. A total recovery was observed 7 h post-injection. Injection of sterile water in the contralateral eye failed to modify the ERG waveform (bottom traces). (B) Response profile of the SKF38393 action on the ERG b-wave amplitude as a function of time. Arrows indicate the time at which the ERGs presented in panel A were selected. The vertical dotted line represents the injection time. (C) Results from twenty-four injections of SKF38393 ($1 \text{ mg}(100 \mu\text{l})^{-1}$) and of the vehicle were pooled together and normalized. A significant decrease was observed during 2 h post-injection. One hundred percent level represents the mean amplitude before injection. Error bars represent SEM. In this and all figures: * = $p < 0.1$, ** = $p < 0.05$, *** = $p < 0.001$.

lack of changes of the ERG b-wave amplitude for the control eye throughout the recording period. Pooled data from 24 SKF38393 injections were normalized and results are shown in panel C of Fig. 1 (a significant effect was observed in 23 out of the 24 injections. In this and all subsequent cases, data from all injections were used for statistical analysis). The maximum inhibitory effect of the DA agonist was observed 2 h post-injection, with a mean reduction of $34.0\% \pm 4.1\%$ ($t_{\text{paired}} = -3.66$, $p = 0.001$). The difference in b-wave amplitude between SKF38393 and control eyes returned to control values 7 h post-injection ($t_{\text{paired}} = -1.94$, $p = 0.06$). The action of SKF38393 ($1 \text{ mg}(100 \mu\text{l})^{-1}$) on the ERG b-wave was comparable in all conditions of illumination (oneway ANOVA, $f_{(22,2)} = 1.51$, $p = 0.24$). The reduction of the b-wave amplitude was $26.8\% \pm 8.0\%$ ($n = 6$), $45\% \pm 6.8\%$ ($n = 10$), and $34.6 \pm 5.1\%$ ($n = 9$) in photopic, mesopic, and scotopic conditions, respectively.

Two SKF38393 doses were used in the study, 0.1 and $1 \text{ mg}(100 \mu\text{l})^{-1}$. When compared to the vehicle, the low concentration yielded a mean decrease of $20.3\% \pm 6.1\%$ ($n = 6$) of the b-wave amplitude, while the high concentration led to a reduction of $34.0\% \pm 4.1\%$ ($n = 24$). Even if only two doses were studied, the effect of SKF38393 appears to be dose-dependent since a higher concentration of the agonist yielded a more pronounced reduction of the b-wave (Kruskal–Wallis, $p = 0.0001$). Only the $1 \text{ mg}(100 \mu\text{l})^{-1}$ dose produced an effect that was statistically significant ($t_{\text{paired}} = -3.66$, $p = 0.001$).

The D_1 -like receptor agonist A77636 had a stronger effect on the b-wave than SKF38393. Three representative examples are shown in Fig. 2. Panel A presents the effect of A77636 at a dose of $1 \text{ mg}(100 \mu\text{l})^{-1}$ in the mesopic lighting condition (filled symbol). The agonist considerably reduced the b-wave amplitude 8 h post-injection (decrease of 60% from pre-injection recordings), whereas the vehicle had no effect in the control eye (open symbol). As illustrated, no recovery of the b-wave was observed at this dose of A77636. This observation stands for all five injections made at 1 mg despite the fact that post-injection recordings lasted up to 18 h. In all five cases the b-wave decrease was significant (Wilcoxon, $p = 0.043$) 2 h after the drug administration, and reached a value of $60.3 \pm 4.6\%$ after 10 h. Panel B shows the action of A77636 administered at $0.5 \text{ mg}(100 \mu\text{l})^{-1}$. In this case, the reduction of the b-wave was of 51.0% after 3 h. When considering all four injections at this dose, A77636 produced a reduction of $43.0\% \pm 6.5\%$ of the b-wave amplitude after 3 h. Two out of four injections recovered after 10 h, the two others failed to recover. Finally, A77636 was injected at a low concentration of $0.2 \text{ mg}(100 \mu\text{l})^{-1}$ in one animal ($n = 2$). In both cases, the agonist failed to modify the amplitude of the ERG (see panel C).

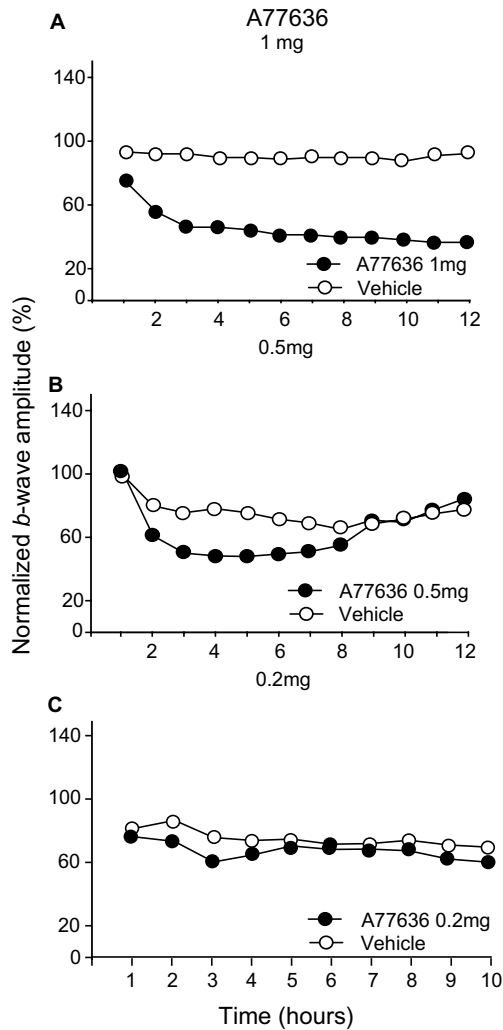


Fig. 2. Effects of the administration of the D_1 -like agonist A77636 at different doses in mesopic condition. (A) $1 \text{ mg}(100 \mu\text{l})^{-1}$; (B) $0.5 \text{ mg}(100 \mu\text{l})^{-1}$; $0.2 \text{ mg}(100 \mu\text{l})^{-1}$. Only the first two doses yielded a decrease of the ERG b-wave. Results in panels A–C come from three animals.

Blocking the D_1 -like receptors with the antagonist SCH23390 ($1 \text{ mg}(100 \mu\text{l})^{-1}$) also produced a reduction of $28.9\% \pm 8.8\%$ of the b-wave amplitude 3 h post-injection in the mesopic condition (panel A of Fig. 3). The amplitude of the b-wave was significantly different from that of the control eye 1 h after injection (Wilcoxon, $p = 0.028$) and the reduction in signal amplitude lasted for the next 6 h. A significant effect of SCH23390 on the b-wave amplitude was observed in four out of six injections.

3.2. D_2 -like receptor contribution to b-wave amplitude

Injections of the D_2 -like receptor agonist NPA failed to produce any significant changes in the b-wave amplitude. Panel A of Fig. 4 shows the outcome of twelve injections:

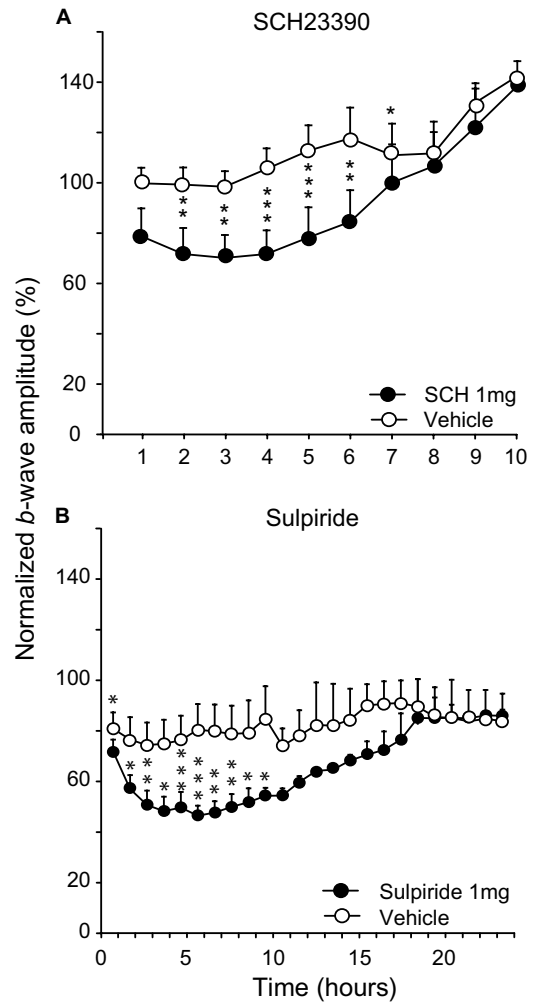


Fig. 3. (A) Effects of the D_1 -like receptor antagonist SCH23390 ($1 \text{ mg}(100 \mu\text{l})^{-1}$). The injection of this ligand ($n = 6$) caused a significant reduction of b-wave amplitude from 2 to 6 h post-injection. Results from six injections in mesopic condition were pooled. (B) Effect of the injection of the D_2 -like receptor antagonist sulpiride $1 \text{ mg}(100 \mu\text{l})^{-1}$ ($n = 8$) in the mesopic condition. The ligand produced a reduction that was maximal 6 h after injection. Error bars represent SEM.

clearly, there was no significant difference between the b-wave amplitude of the NPA-treated and normal eye up to 10 h after the injections. Increasing or decreasing the NPA concentration yielded comparable findings. This is illustrated in panel B of Fig. 4. Varying the dose from 0.0001 to $1 \text{ mg}(100 \mu\text{l})^{-1}$ failed to produce any significant changes in the waveform amplitude ($1 \text{ mg}(100 \mu\text{l})^{-1}$, mean reduction of the b-wave $27\% \pm 1.8\%$, $n = 9$; $0.1 \text{ mg}(100 \mu\text{l})^{-1}$, $20.4 \pm 6.5\%$, $n = 8$; $0.01 \text{ mg}(100 \mu\text{l})^{-1}$, 16.7% , $n = 1$; $0.001 \text{ mg}(100 \mu\text{l})^{-1}$, $22.0 \pm 1.6\%$, $n = 4$; $0.0001 \text{ mg}(100 \mu\text{l})^{-1}$, $-0.08 \pm 1.8\%$, $n = 3$) (Kruskal–Wallis, $p = 0.12$). Similarly, changing the background luminance did not reveal any notable physiological impact of NPA (Kruskal–Wallis, $p = 0.73$). The numbers of injections of NPA $1 \text{ mg}(100 \mu\text{l})^{-1}$ in the photopic, mesopic and scotopic conditions were respectively 4, 4, and 1.

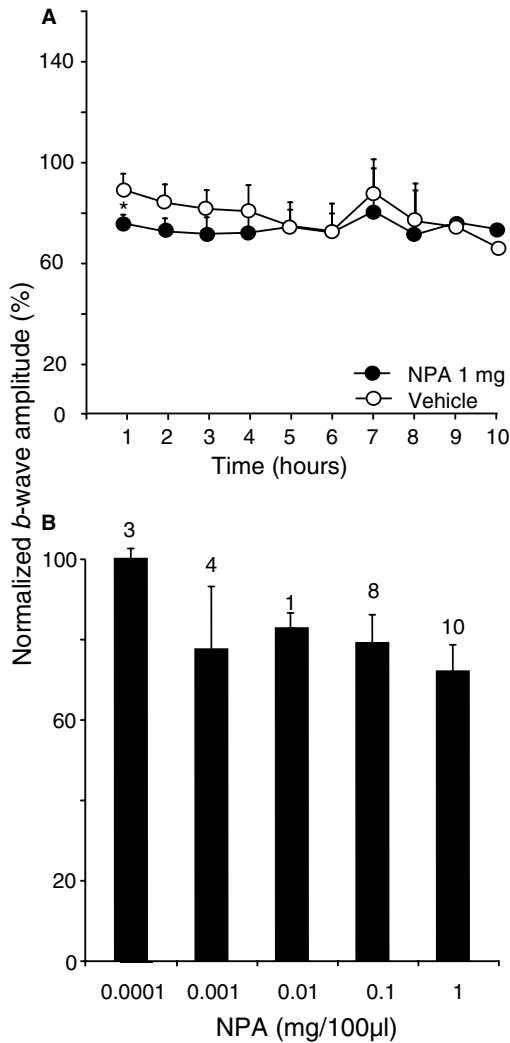


Fig. 4. (A) Injection of the D₂-like receptor agonist Norpropylapomorphine (NPA) (1 mg(100 µl)⁻¹) failed to modify the b-wave amplitude. Number of injections was 9. (B) Effect of various concentrations of NPA (1 to 0.0001 mg(100 µl)⁻¹) on the b-wave. Doses greater than 0.0001 mg(100 µl)⁻¹ tended to reduce the ERG. However, no dose was found to produce a significant change of the waveform amplitude. The number of injections is shown at the top of each bar. Error bars represent SEM.

A different picture emerged when the D₂-like receptor antagonist sulpiride was administered (panel B of Fig. 3). This ligand reduced the b-wave amplitude for more than 15 h. The maximum effect was seen 6 h post-injection and the b-wave amplitude was reduced to $59.8 \pm 4.6\%$ of the original amplitude (Wilcoxon, $p = 0.018$).

4. Discussion

The present findings indicate that retinal activity, as measured with the b-wave of the electroretinogram, is mainly modulated by the activation of D₁-like rather

than D₂-like receptors. There was no relationship between the observed changes and the various luminance conditions in which the testing was done, suggesting that the effective ligands studied here were not specific to the rod or cone pathways of the retina.

The D₁-like receptor agonists SKF38393 and A77636 produced a significant reduction in the amplitude of the b-wave, but not of the a-wave (data not shown). A similar modulation of the ERG was reported by Textorius et al. (1989) who demonstrated that DA provoked a decrease of the ERG b-wave in a rabbit in vivo eye cup preparation. Comparable findings were described after the systemic administration of apomorphine in the same animal model (Jagadeesh & Sanchez, 1981). To our knowledge, only one study previously investigated the impact of SKF38393 on retinal evoked potentials. In rabbits, Marmor et al. (1988) reported that an intramuscular injection SKF38393 had very little inhibitory effect on the b-wave amplitude and oscillatory potentials. The fact that they observed a more subtle effect than ours is likely due to the difference in the mode of administration of the ligand (intramuscular vs. intravitreal). We are not aware of any study reporting the effect of the agonist A77636 on retinal function. On the basis of its long-lasting action on the b-wave amplitude, this ligand appears to be more potent than SKF 38393 at similar doses. Our findings that D₁-like agonist can modulate the amplitude of the ERG b-wave are further substantiated by the observation that, in monkey, the administration of the D₁-like agonist cy208-243 reduces the pattern ERG at low spatial frequencies (Peppe, Antal, Tagliati, Stanzone, & Bodis-Wollner, 1998). The mechanisms by which the D₁-like agonists used in the present study affect the ERG remain to be determined. It is generally accepted that the b-wave reflects the K⁺-mediated depolarization of Müller cells (Newman & Odette, 1984; Wen & Oakley, 1990; Xu & Karwoski, 1994) even though there is mounting evidence that depolarizing ON bipolar cells may also directly contribute to the waveform (Green & Kapousta-Bruneau, 1999; Stockton & Slaughter, 1989). Both cell types contain D₁-like receptors (Nguyen-Legros et al., 1987; Nguyen-Legros, Simon, Caille, & Bloch, 1997; Veruki & Wässle, 1996) and may therefore contribute to the effects of the D₁-like agonists.

While the administration of D₁-like agonists induced a decrease of the ERG b-wave, the D₂-like agonist studied here (R(-)-NPA; affinity 100- to 425-fold greater than for D₁-like receptors, Baldessarini et al., 1994; Gao et al., 1990) failed to produce a significant change in the amplitude of the ERG. This is somewhat surprising given that apomorphine, a D₂-like agonist, is known to reduce the amplitude of the ERG b-wave. Recent studies showed that apomorphine has a greater affinity for D₂ than D₁-like receptors 2.6- to 22-fold depending

on the enantiomer¹ and nature of the non-retinal brain tissue studied (Baldessarini et al., 1994; De Keyser et al., 1995; Gao et al., 1990). One explanation would be that, in the retina, apomorphine binds preferentially to D₁-like receptors. Another explanation would be that NPA may not target receptors coupled with b-wave mechanisms. This latter suggestion is however unlikely because Müller cells, which are implicated in b-wave genesis, possess D₂ receptors (Biedermann, Frohlich, Grosche, Wagner, & Reichenbach, 1995; in the guinea pig retina). If present in rabbits, it is possible that activation of these receptors yielded subtle changes that may not be seen at the level of a gross retinal response such as the ERG, or simply that NPA acts on D₂-like receptors other than the kind Müller cells express (e.g. D₄). Another possibility may come from the fact that the presynaptic autoreceptors are of the D₂-like type. Activation of the autoreceptors would reduce the synthesis and release of the endogenous DA which normally binds to both D₁- and D₂-like receptors (Stanzione et al., 1999), thus masking any strong effect of the ligand. NPA could have a dual action, inhibiting DA-release through the D₂-like autoreceptors but simultaneously activating b-wave reduction through postsynaptic D₁-like receptors. Although not statistically significant, the reduction in b-wave amplitude by NPA at all but the lowest dose (Fig. 4B) is consistent with the last assumption, i.e. the D₂-like effect outweighing the D₁-like effect.

Overall, given the high affinity of the DA agonists SKF38393 and A77636 that we used for the D₁-like receptors (150- and 30- to 750-fold greater for D₁-like receptors, respectively; Keabian et al., 1992; Seeman & Van Tol, 1994), the results of the present study strongly suggest that the effect of DA on the ERG described by Jagadeesh and Sanchez (1981) and Textorius et al. (1989) was most likely mediated through activation of D₁-like receptors.

The intravitreal injection of the D₁-like antagonist SCH23390 (affinity: 5500-fold; (Seeman & Van Tol, 1994)) produced a significant reduction of the b-wave amplitude, but less pronounced than that induced by the agonist SKF38393. This result was unexpected. Indeed, blocking the D₁-like receptors should yield a reduction of the sites available for DA, therefore mimicking a decrease of DA content. This decrease would then yield an increase of the b-wave amplitude (Lafond et al., 1994; Olivier et al., 1986; Olivier et al., 1987). Marmor et al. (1988) found the same contradictory effect in rabbits as they reported that the intramuscular injection of both SCH23390 and SKF38393 reduced

the b-wave amplitude. Other laboratories also found that D₁ antagonists can modulate retinal responses. Wioland, Rudolf, and Bonaventure (1990) showed that haloperidol, a mixed D₁- and D₂-like antagonist, reduced the b-wave amplitude of the chicken retina in photopic condition. In the isolated, arterially perfused eye of the cat, the D₁-like antagonist fluphenazine provoked an opposite effect, i.e. an enhancement of the b-wave (Schneider & Zrenner, 1991). In the present study, SCH23390 was administered at a single dose of 1 mg(100 µl)⁻¹. It may be possible that at this dose, the ligand exhibits some neurotoxicity. This assumption however can hardly be reconciled with the fact that there was a recovery of the ERG waveform following the antagonist's administration. Another option is that SCH23390 exerted its action via non-DA receptors. For example, it has been reported that NPA not only has affinity for D₁- and D₂-like receptors but also for alpha-2 adrenoreceptors (Baldessarini et al., 1994). Clearly, the mechanisms by which SCH23390 can modify the retinal function remain to be determined.

While the D₂-like agonist NPA had no significant effect on the ERG, the antagonist *S*-sulpiride (affinity 3000-fold for D₂-compared to D₁-like receptors, (Seeman & Van Tol, 1994)) provoked a long-lasting reduction of the b-wave amplitude. Again, other studies revealed that D₂-like antagonists can alter retinal physiology. In rabbits, Jagadeesh, Lee, and Salazar-Bookman (1980) reported that the intravenous injection of chlorpromazine increased the amplitude of the b-wave. This effect is opposite to that described in the present study. It may be due to differences in the mode of administration or in the specificity of the two agents at different receptor subtypes. In cats, the administration of sulpiride induced an increase of the b-wave amplitude in scotopic conditions (Schneider & Zrenner, 1991). This is contrary to our findings and the difference may again come from inter-species differences or from the distinct experimental approach (in vivo vs. isolated eye preparation). It is possible that the reduction of b-wave amplitude that was observed here after the administration of sulpiride may be mediated through the DA autoreceptors. Blocking the presynaptic autoreceptors could raise the synthesis and release of the endogenous DA, and the released DA could then activate both postsynaptic D₁- and D₂-like receptors.

In conclusion, our data further support the involvement of DA in the modulation of retinal function. They showed that the dopaminergic impact on the ERG b-wave is largely mediated by D₁-like receptors, suggesting that the latter are preferentially involved in the transmission of information within the inner and middle parts of the retina. Since none of the agonists used here has an exclusive affinity for D₁- or D₂-like receptors, and that D₂-like antagonist can alter the amplitude of the b-wave, we cannot rule out any subtle contribution of

¹ Note that the enantiomer of apomorphine used by Jagadeesh and Sanchez (1981) was not specified in their article.

D₂-like receptors in the dopaminergic modulation of the ERG.

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