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Effect of a cGMP-specific phosphodiesterase inhibitor on retinal function

Marielle Estrade [a], Pascal Grondin [b], Jacques Cluzel [a], Brigitte Bonhomme [a], Michel Doly [a,*]

[a] *Laboratoire de Biophysique Sensorielle, Facultés de Médecine et de Pharmacie, 28, Place Henri Dunant, 63001 Clermont-Ferrand, France*
[b] *Laboratoire Glaxo Wellcome, Centre de Recherches, 25 Avenue du Québec, 91951 Les Ulis, France*

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Abstract

Multiple forms of phosphodiesterase have been reported in many tissues. Phosphodiesterase 6, a cGMP-specific phosphodiesterase, is described as a photoreceptor cell-specific phosphodiesterase. Phosphodiesterase 6 is known to play a crucial role in visual function. A novel phosphodiesterase inhibitor, GF248 (5[″(propoxy),7′(4-morpholino)-phenacyl],[1-methyl-3 propyl]pyrazolo[4,3*d*]pyrimidin-7-one), has been described to be a very potent cGMP-specific phosphodiesterase inhibitor. In the present study, we compared the potency of GF248 and other known cGMP-specific phosphodiesterase inhibitors on phosphodiesterase 5 and phosphodiesterase 6. GF248 displayed an IC_{50} of 2 and 5 nM for phosphodiesterase 5 and phosphodiesterase 6, respectively. Thereafter, we assessed the effect of GF248 on retinal function, using an ex vivo model of isolated retina electroretinogram recording. Exposure of retina to GF248 resulted in a dose-dependent decrease in electroretinogram amplitude (PIII and b-waves), with no marked modification of PIII and b-wave implicit time. Among other phosphodiesterase inhibitors, DMPPO (1,3-dimethyl-6-(2-propoxy-5-methanesulfonylamidophenyl)pyrazolol[3,4*d*]-pyrimidin-4-(5*H*)-one) and dipyridamole, cGMP-specific phosphodiesterase inhibitors, and IBMQ (1-isobutyl-3-methylimidazol[1,5-*a*]quinoxalin-4-(5*H*)one), a nonselective phosphodiesterase inhibitor, altered retinal function but less potently than GF248, consistent with their in vitro phosphodiesterase 6 inhibition. Phosphodiesterase 3- and phosphodiesterase 4-selective inhibitors, cilostamide and rolipram, respectively, did not affect retinal function at 10 μmol l^{-1}. Our conclusion from these data is that GF248, a potent phosphodiesterase 6 inhibitor, could interfere with visual transduction by cGMP accumulation.

Keywords: GF248; Phosphodiesterase inhibitor; Electroretinography; Retina, isolated; (Rat)

1. Introduction

Guanosine 3′:5′-cyclic guanosine monophosphate (cGMP) is a key messenger involved in the phototransduction process of rod outer segments of the mammalian retina. Its metabolism is, thus, critically important for visual function. The cGMP concentration in retinal rods depends on the activity of 2 enzymes: guanylate cyclase, which is mainly regulated by the intracellular Ca^{2+} level, and cGMP phosphodiesterase, which is controlled by the light-dependent enzyme cascade. The cGMP level is high in darkness, resulting in the opening of cation channels, and decreases upon illumination, resulting in the closure of cation channels, interruption of the flow of sodium and calcium into the cell and hyperpolarization of the plasma membrane (for recent reviews, see Palczewski, 1994; Bownds and Arshavsky, 1995; Yarfitz and Hurley, 1994).

Multiple forms of phosphodiesterases have been isolated from many tissues. There are at least 7 isoenzymes of phosphodiesterase classified by Sonnenburg and Beavo (1994) according to their primary protein and cDNA sequences, substrate specificity and differential sensitivity to regulation by endogenous (calmodulin and other cyclic nucleotides) and exogenous (selective inhibitors) modulators: a calcium–calmodulin-dependent phosphodiesterase (phosphodiesterase 1), a cGMP-stimulated phosphodiesterase (phosphodiesterase 2), a cGMP-inhibited phosphodiesterase (phosphodiesterase 3), a cAMP-specific phosphodiesterase (phosphodiesterase 4), a cGMP-specific phosphodiesterase (phosphodiesterase 5), a photoreceptor cGMP-specific phosphodiesterase (phosphodiesterase 6) and a cAMP-specific rolipram-insensitive phosphodiesterase (phosphodiesterase 7). Two of them selectively

* Corresponding author. Tel.: +33-4-7360-8015; fax: +33-4-7326-8818; e-mail: michel.doly@cjp.u-clermont1.fr

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hydrolyse cGMP: phosphodiesterase 5 and phosphodiesterase 6 (Beavo and Reifsnyder, 1990).

A number of selective inhibitors of the phosphodiesterase isoenzymes have been described and some of them are used clinically. However, relatively little information has been reported about phosphodiesterase 6. Recently, a new selective cGMP-specific phosphodiesterase inhibitor, called GF248 (5[″(propoxy),7′(4-morpholino)-phenacyl],[1-methyl-3 propyl]pyrazolo[4,3*d*]pyrimidin-7-one), has been described. It belongs to a family of pyrazolopyrimidinone compounds which are potentially useful in the treatment of cardiovascular disorders such as angina, hypertension, heart failure and atherosclerosis (Bell and Terret, 1993). The present study was done, first, to compare the effect of GF248 and other known cGMP-specific phosphodiesterase inhibitors on phosphodiesterase 5 and phosphodiesterase 6 in vitro. Then, we studied the influence of this new inhibitor on retinal function and compared its effect with the effect of other phosphodiesterase inhibitors, i.e., DMPPO (1,3-dimethyl-6-(2-propoxy-5-methanesulfonylamidophenyl)pyrazolol[3,4*d*]-pyrimidin-4-(5*H*)-one), IBMQ (1-isobutyl-3-methylimidazol[1,5-*a*]quinoxalin-4-(5*H*)one), dipyridamole, rolipram (4-(3-cyclopentyloxy-4-methoxyphenyl)2-pyrrolidone) and cilostamide (*N*-cyclohexyl-*N*-methyl-4-(1,2-dihydro-2-oxo-6-quinolyloxy)butyramide) in the same model. In this paper, we evaluated the potential effects of these inhibitors on retinal function by recording electroretinograms of the specific electrophysiological signal whose generation implies the activation of the phototransduction process.

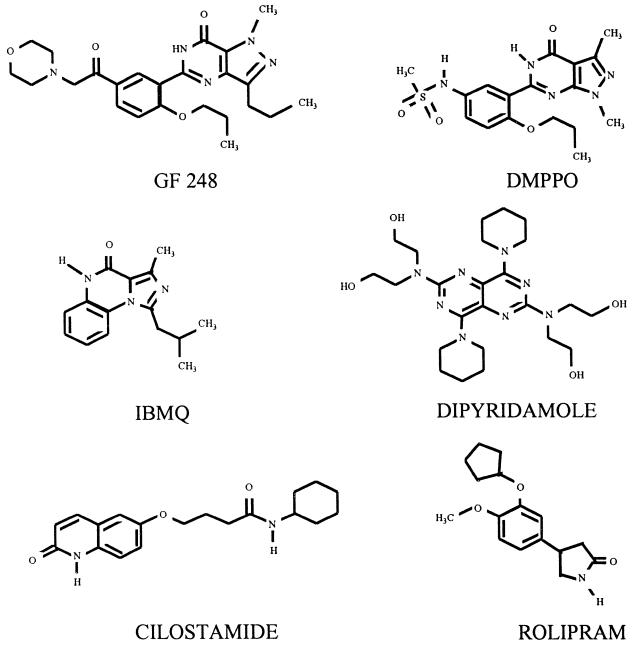

Fig. 1. Chemical structure of phosphodiesterase inhibitors.

2. Materials and methods

2.1. Materials

Phosphodiesterase inhibitors were provided by Glaxo Wellcome Laboratory (Les Ulis, France) (Fig. 1).

2.2. Phosphodiesterase assay

Phosphodiesterase 5 from bovine aorta was obtained and its phosphodiesterase activity was assayed as previously described (Coste and Grondin, 1995). Phosphodiesterase 6 from bovine retina was obtained as described in Sitaramayya et al. (1977) and kindly supplied by N. Virmaux (Inserm U338, Strasbourg).

2.3. Isolated retina model

The experimental setup was described in details by Doly et al. (1980). Female Wistar rats (300 to 350 g) were dark-adapted for at least 2 h before being killed. One eye was removed, cleaned of conjunctival tissue and dissected at the equator. Retinas were progressively detached from the pigment epithelium, then released by cutting at the level of the optic nerve. The dissection was performed in perfusion liquid with the following composition: NaCl, 121.5 mmol l^{-1}; KCl, 3.1 mmol l^{-1}; $CaCl_2$, 0.4 mmol l^{-1}; $MgSO_4$, 0.6 mmol l^{-1}; $NaHCO_3$, 22.4 mmol l^{-1}; NaH_2PO_4, 0.5 mmol l^{-1}; sodium glutamate, 0.5 mmol l^{-1}; glucose 28 mmol l^{-1}; human plasma 3%. The retina was then mounted in a cell perfused by the perfusion liquid, maintained at 37°C and permanently bubbled by a mixture of 95% O_2 and 5% CO_2.

On the day of experiment, GF248 was solubilized in 1‰ of NaOH 1N and added to 500 ml of perfusion medium in order to obtain final concentrations of 0.01, 0.1, 0.5, 1 and 10 μmol l^{-1}. For control experiments, the perfusion medium was supplemented with 1‰ of 1 M NaOH. Experiments were performed with 6 retinas for each concentration tested.

DMPPO, IBMQ, dipyridamole, rolipram and cilostamide were presolubilized in a small volume of perfusion medium and added to 500 ml of perfusion medium at the final concentration of 10 μmol l^{-1}. Experiments were performed with 5 retinas for each inhibitor tested.

2.4. Electroretinogram recording

The retina was illuminated by a brief flash of white light (300 lx, 1 ms) every 5 min. The difference in potential between 2 Ag electrodes located on either side of the retina was amplified, processed and stored. Experiments were conducted in a dark room lit by a dim red light (wavelength > 620 nm) and procedures were fully automated.

The white flash stimulus applied to an isolated retina generates a transretinal potential which is recorded on an electroretinogram. By repeating the same stimulus at regular intervals, we observed a reproducible evolution of wave amplitude. By plotting the amplitude of the waves vs. time, we obtained a pattern that we called the survival curve. This pattern could be described as follows: first, an increase due to the adaptation of the retina to the survival conditions, then a stationary phase, and finally a progressive decrease corresponding to the end of survival (Doly et al., 1994). The different phosphodiesterase inhibitors were added to the perfusion solution at the start of the stationary phase and PIII- and b-waves were monitored for the rest of the survival.

2.5. Statistical analysis

The amplitude and implicit time values of PIII- and b-waves, 30 min and 2 h after addition of GF248 to perfusion medium, were statistically analyzed. For the other compounds, only the amplitude of PIII- and b-waves was statistically analyzed. The mean values were compared by Student's *t*-test.

3. Results

3.1. Comparison of the effect of GF248 on phosphodiesterase 5 and phosphodiesterase 6 activities in vitro

As shown in Table 1, GF248 displayed comparable activity on phosphodiesterase 5 and phosphodiesterase 6, with IC_{50} values of 2 and 5 nM, respectively. DMPPO was 4-fold less potent on phosphodiesterase 6 than GF248 and displayed a 7-fold selectivity for phosphodiesterase 5 vs. phosphodiesterase 6. Dipyridamole appeared to be a very poor phosphodiesterase 5 and phosphodiesterase 6 inhibitor. IBMQ, a nonspecific phosphodiesterase inhibitor, was about 10- and 50-fold less potent than GF248 on phosphodiesterase 5 and phosphodiesterase 6, respectively.

Table 1
Inhibition of different cGMP-specific phosphodiesterase isoforms by phosphodiesterase inhibitors

Compounds	IC_{50} (nM)	
	Phosphodiesterase 5	Phosphodiesterase 6
GF248	2 ± 1	5 ± 2
DMPPO	3 ± 1	22 ± 5
Dipyridamole	920 ± 50	1100 ± 70
IBMQ	25 ± 5	250 ± 50

Phosphodiesterase activities were determined as described in Section 2. IC_{50} values represent the mean $\pm$ S.D. of at least three independent determinations in which dose–response curves were obtained for compound concentrations ranging from 1 nM to 10 μM.

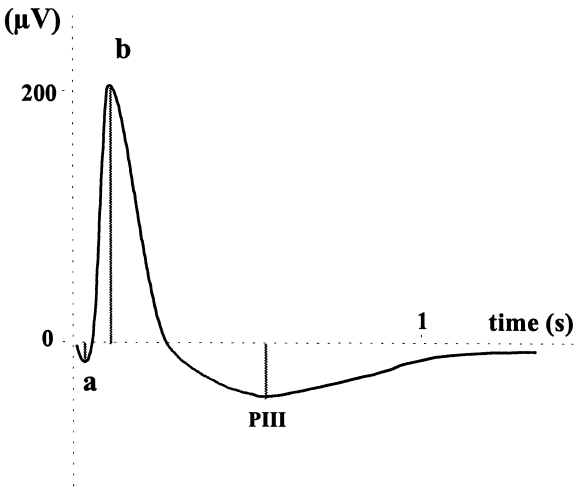

Fig. 2. Example of electroretinogram recorded on isolated rat retina to light stimulation (white stimulation, 300 lx, 1 ms), showing the b-wave of the a-wave to the positive b-wave peak. The amplitude of the PIII-wave was measured from the basal line to the trough of the PIII-wave. Implicit times of the b- and PIII-waves were measured from the stimulus onset to the maximum of the potential under consideration.

From these data, GF248 appeared to be the most potent phosphodiesterase 6 inhibitor for further studies.

3.2. Effect of GF248 on visual function

The electroretinogram displays 3 waves (Fig. 2): an initial negative a-wave, a positive b-wave and a slow negative PIII-wave which can be compared to the PIII component of Granit electroretinogram analysis (Granit, 1933). The electroretinogram a- and PIII-waves reflect hyperpolarization of the rod outer segment, which follows the activation of the phototransduction process by light. The b-wave is generated by a K^+ current through Müller cells (Miller and Dowling, 1970).

3.2.1. PIII-wave

Fig. 3 shows the dose-related effect of GF248 on the electroretinogram PIII-wave amplitude. Immediately after addition of GF248 at 0.5, 1 and 10 μmol l^{-1}, we observed a rapid and significant decrease in the PIII-wave amplitude as compared to that of the control retinas. Statistical analysis of the wave amplitudes is presented Table 2. Thirty minutes after the addition of GF248, the PIII-wave amplitude was nearly 60%, 40% and 20% of its initial value for concentrations of 0.5, 1 and 10 μmol l^{-1}, respectively. In the control experiment, at this time, no decrease of PIII-wave amplitude was observed.

Two hours after GF248 administration at 1 and 10 μmol l^{-1}, the PIII-wave amplitude was significantly different from the control amplitude. For the other concentrations (0.5, 0.1 and 0.01 μmol l^{-1}), no significant difference was observed between treated and control retinas.

We observed a significant decrease in the implicit time, 30 min after addition of GF248 at 0.1 or 0.5 μmol l^{-1}

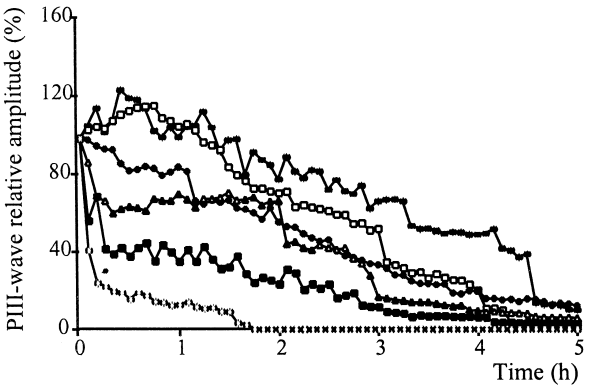

Fig. 3. Evolution of the relative amplitude of the electroretinogram PIII-wave in the presence of GF248 at different concentrations (for each concentration: $n = 6$) (●: control, □: GF248 = 0.01 μmol l^{-1}, ◆: GF248 = 0.1 μmol l^{-1}, ▲: GF248 = 0.5 μmol l^{-1}, ■: GF248 = 1 μmol l^{-1}, ○: GF248 = 10 μmol l^{-1}). On the *y*-axis, the value 100 represents the average of the PIII-wave amplitude at the time of injection.

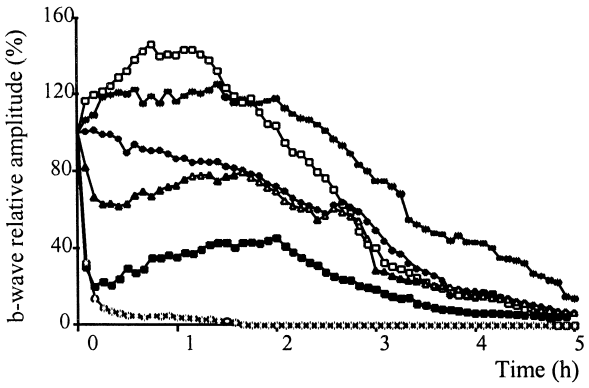

Fig. 4. Evolution of the relative amplitude of the electroretinogram b-wave in the presence of GF248 at different concentrations (for each concentration: $n = 6$) (●: control, □: GF248 = 0.01 μmol l^{-1}, ◆: GF248 = 0.1 μmol l^{-1}, ▲: GF248 = 0.5 μmol l^{-1}, ■: GF248 = 1 μmol l^{-1}, ○: GF248 = 10 μmol l^{-1}). On the *y*-axis, the amplitude at the time of injection.

(Table 2), but no change at the higher concentrations. Nevertheless, the electroretinograms were so distorted under the latter conditions that the determination of the implicit time became inaccurate. Two hours after the addition of GF248, no difference in implicit time appeared between control and treated retinas.

3.2.2. b-Wave

Fig. 4 shows the effect of GF248 on the electroretinogram b-wave amplitude and statistical analysis of amplitude is presented in Table 3. Addition of GF248 at 0.5, 1 and 10 μmol l^{-1} to the perfusion medium induced a rapid and significant decrease of the electroretinogram b-wave amplitude. Thirty minutes after the addition of GF248, the b-wave amplitude was 63%, 30% and 5% of its initial value for concentrations of 0.5, 1 and 10 μmol l^{-1}, respectively. No significant decrease in amplitude was observed for GF248 at the concentrations 0.01, 0.1 μmol l^{-1} and for the control experiment.

Two hours after the addition of GF248 at 1 and 10 μmol l^{-1}, the b-wave amplitude was significantly different from the b-wave amplitude of control retinas. At the concentrations 0.1 and 0.5 μmol l^{-1}, the b-wave amplitude decreased slowly. However, no significant difference was observed. No effect was observed when GF248 was added at 0.01 μmol l^{-1}. The survival curves of b-wave amplitude showed that the decrease in b-wave amplitude was proportional to the GF248 concentration used.

Statistical analysis of the influence of GF248 on the implicit time of the b-component demonstrated that there was no significant modification of the implicit time of the b-component.

3.3. Comparative effect of various phosphodiesterase inhibitors on visual function

Table 4 summarized the effects of several phosphodiesterase inhibitors at 10 μmol l^{-1} on the electroretino-

Table 2
Statistical analysis (*t*-test) of amplitude and implicit time of the PIII-component

GF 248 concentration (μM)	30 min after addition of GF 248		2 h after addition of GF 248	
	Relative amplitude[d] (%)	Relative implicit time[d] (%)	Relative amplitude[d] (%)	Relative implicit time[d] (%)
0	121 ± 25	101 ± 7	79 ± 7	97 ± 9
0.01	115 ± 6 (NS)	109 ± 7 (NS)	72 ± 11 (NS)	102 ± 10 (NS)
0.1	84 ± 9 (NS)	85 ± 7[a]	66 ± 14 (NS)	94 ± 32 (NS)
0.5	64 ± 6 (NS)	86 ± 10[a]	67 ± 11 (NS)	80 ± 13 (NS)
1	38 ± 6[a]	90 ± 10 (NS)	24 ± 6[c]	87 ± 12 (NS)
10	16 ± 3[b]	88 ± 12 (NS)	No ERG[c]	No ERG[c]

The data represent the means ± S.E.M. NS denotes no significant difference vs. control.
[a] $P < 0.05$ vs. control.
[b] $P < 0.01$ vs. control.
[c] $P < 0.001$ vs. control.
[d] The value 100 represents the average of the PIII-wave amplitude or implicit time at the time of injection.

Table 3
Statistical analysis of amplitude and implicit time of the b-component

GF 248 concentration (μM)	30 min after addition of GF 248		2 h after addition of GF 248	
	Relative amplitude[d] (%)	Relative implicit time[d] (%)	Relative amplitude[d] (%)	Relative implicit time[d] (%)
0	120 ± 9	100 ± 5	118 ± 18	98 ± 7
0.01	132 ± 7 (NS)	98 ± 3 (NS)	104 ± 19 (NS)	95 ± 5 (NS)
0.1	90 ± 12 (NS)	99 ± 3 (NS)	72 ± 9 (NS)	96 ± 7 (NS)
0.5	63 ± 12[b]	96 ± 5 (NS)	70 ± 14 (NS)	94 ± 4 (NS)
1	30 ± 7[c]	98 ± 6 (NS)	46 ± 16[a]	97 ± 5 (NS)
10	5 ± 2[c]	92 ± 8 (NS)	No ERG	No ERG

The data represent the means ± S.E.M. NS denotes no significant difference vs. control.
[a] $P < 0.05$ vs. control.
[b] $P < 0.01$ vs. control.
[c] $P < 0.001$ vs. control.
[d] The value 100 represents the average of the b-wave amplitude or implicit time at the time of injection.

Table 4
Comparative effects of PIII-component amplitude after exposure of isolated retinas to several phosphodiesterase inhibitors

Inhibitor tested	Concentration (μM)	Relative amplitude[d] (%)	
		30 min after addition of inhibitor	2 h after addition of inhibitor
Control		88 ± 14	54 ± 11
Cilostamide	10	98 ± 5 (NS)	56 ± 7 (NS)
Rolipram	10	92 ± 5 (NS)	62 ± 11 (NS)
IBMQ	10	77 ± 18 (NS)	No ERG[c]
GF248	10	16 ± 4[c]	No ERG[c]
DMPPO	10	34 ± 9[a]	No ERG[c]
Dipyrydamole	10	118 ± 15 (NS)	76 ± 23 (NS)

The data represent the means ± S.E.M. NS denotes no significant difference vs. control.
[a] $P < 0.05$ vs. control.
[c] $P < 0.001$ vs. control.
[d] The value 100 represents the average of the PIII-wave amplitude at the time of injection.

gram PIII-wave amplitude. Cilostamide, rolipram and dipyridamole did not have a significant effect on the PIII-wave amplitude at any time. Thirty minutes after addition of DMPPO or GF248 to the perfusion medium, PIII-wave amplitude showed a significant decrease as compared to the PIII-wave amplitude of control retinas. The effect of GF248 was more marked than that of DMPPO. Thirty minutes after addition of IBMQ, no modification of the PIII-wave amplitude was observed.

Two hours after addition of DMPPO, IBMQ and GF248, the PIII-wave amplitude was completely abolished. In the control experiment, at this time the PIII-wave amplitude was nearly 50% of its initial value.

Table 5 presents the effects of several phosphodi-

Table 5
Comparative effects of b-wave amplitude after exposure of isolated retinas to several phosphodiesterase inhibitors

Inhibitor tested	Concentration (μM)	Relative amplitude[d] (%)	
		30 min after addition of inhibitor	2 h after addition of inhibitor
Control		108 ± 13	98 ± 12
Cilostamide	10	100 ± 10 (NS)	52 ± 24 (NS)
Rolipram	10	95 ± 11 (NS)	47 ± 17 (NS)
IBMQ	10	No ERG[c]	No ERG[c]
GF248	10	5 ± 2[c]	No ERG[c]
DMPPO	10	16 ± 10[b]	No ERG[c]
Dipyridamole	10	120 ± 15 (NS)	97 ± 18 (NS)

The data represent the means ± S.E.M. NS denotes no significant difference vs. control.
[b] $P < 0.01$ vs. control.
[c] $P < 0.001$ vs. control.
[d] The value 100 represents the average of the b-wave amplitude at the time of injection.

esterase inhibitors at 10 μmol l^{-1} on the electroretinogram b-wave amplitude. No modification of the b-wave amplitude was seen 30 min and 2 h after addition of rolipram, cilostamide and dipyridamole.

Thirty minutes after addition of IBMQ, DMPPO and GF248, we observed a significant and drastic decrease of the b-wave amplitude as compared to that of the control retinas. Two hours after addition of these compounds to the perfusion medium, the b-wave was undetectable. In the control retinas, at this time no decrease of the b-wave amplitude was observed.

4. Discussion

The absorption of light by the vertebrate rod outer segment results in a change in the polarization of the plasma membrane of the photoreceptor cell. The key effector of this process is a cGMP-specific phosphodiesterase which regulates, in response to light, the level of a key messenger: cGMP.

In vitro data demonstrate that GF248 is a potent phosphodiesterase 6 inhibitor (5 ± 2 nM). By comparison, DMPPO and dipyridamole, other cGMP-specific phosphodiesterase inhibitors, are less potent inhibitors of phosphodiesterase 6 (22 and 1100 nM, respectively).

Furthermore, this study shows that a cGMP-selective phosphodiesterase inhibitor, GF248, alters the isolated rat retina electroretinogram in a dose-dependent manner when it is added to the perfusion medium.

GF248 acted principally by reducing the amplitude of both the PIII- and b-waves. GF248, which was previously reported to be a selective inhibitor of phosphodiesterase 5 (Bell and Terret, 1993), clearly altered the photoreceptor cGMP-specific phosphodiesterase: phosphodiesterase 6 in retina. Interestingly, phosphodiesterase 5 and phosphodiesterase 6 share a common structure, regulation and substrate-specificity; nevertheless, the cDNA sequence homology between these 2 isoenzymes is less than 25% (Sonnenburg and Beavo, 1994). Our results showed that GF248 acts on a process involved in phototransduction. This compound at 0.01 to 10 μmol l^{-1} caused a dose-related alteration of electroretinogram parameters. We thought that, in GF248-treated retinas, a decrease in cGMP hydrolysis would be associated with a disruption of the signal transduction cascade in rod outer segment. Thus, after a flash of light, the GF248-induced inhibition of light-activated cGMP-phosphodiesterase, coupled with the elevated cGMP level, would prevent closure of all the opened cGMP-activated channels and would thus decrease the sensitivity and the amplitude of the light response. A similar reduction of the amplitude of the PIII- and b-waves has been reported after exposure to phosphodiesterase inhibitors, such as isobutylmethylxanthine (IBMX) (Sandberg et al., 1987), lead (Tessier-Lavigne et al., 1985; Fox and Farber, 1988; Fox et al., 1994), or phosphodiesterase-inhibiting drugs, such as theophylline, caffeine and papaverine hypochloride (Schneider and Zrenner, 1985).

Whatever the concentration used, GF248 did not affect the implicit time of the b-component. At the concentrations of 0.5 and 1 μmol l^{-1}, GF248 decreased the implicit time of the PIII-component. This result is not consistent with the observations of Schneider and Zrenner (1985). These authors reported that the implicit time of the PIII-component showed a dose-dependent increase after injection of phosphodiesterase inhibitors. They hypothesized that the hydrolysis of cGMP might be the rate-limiting step in phototransduction, and that the pharmacological inhibition of this step might be responsible for the observed prolongation of the implicit time. In our case, we could explain the decrease in the implicit time of the PIII-component after addition of GF248 at concentrations 0.5 and 1 μmol l^{-1} as a consequence of a decrease in the wave amplitude and not as a consequence of a physiological event. However, GF248 at a higher concentration did not affect the implicit time, probably because the electroretinograms were distorted. At these concentrations, GF248 had a very marked effect on the visual function, as evidenced in Figs. 3 and 4.

The modification of the sensitivity of the light response is evidenced by the decrease in the amplitude of the PIII- and b-waves. Although phosphodiesterase inhibitors act on the phototransduction process, the effect is always greater on the b-wave amplitude than on the PIII-wave amplitude. This result confirms the observation that the b-wave is the most sensitive parameter of the electroretinogram (Doly et al., 1994). The amplitude of this wave is an accurate measure of the quality of retinal metabolism and synapse connections within the retina.

We also compared the inhibitory activity of GF248 on visual function with the activity of other phosphodiesterase inhibitors. Like GF248, DMPPO and dipyridamole are reported to be potent and specific inhibitors of phosphodiesterase 5 (Bell and Terret, 1993; Coste and Grondin, 1995; Thompson, 1991). However, these inhibitors at a concentration of 10 μmol l^{-1} had different effects on visual function. DMPPO, like GF248, had a dramatic effect on the electroretinogram parameters, which is consistent with the small difference observed on IC_{50} values on phosphodiesterase 6 between the 2 compounds. Furthermore, it was interesting to note that, at the same concentration, GF248 appeared to be more potent than DMPPO, in accordance with their IC_{50}s. At 10 μmol l^{-1}, dipyridamole had no effect on visual function. However, we have shown in a dose-effect study of dipyridamole on electroretinogram parameters that dipyridamole has an effect at a higher concentration: 100 μmol l^{-1} (Estrade et al., 1996). So, it appears that GF248 is a much more potent phosphodiesterase 6 inhibitor than dipyridamole.

Another inhibitor used in this study is IBMQ. This compound displays low selectivity for inhibiting different phosphodiesterases (Coste and Grondin, 1995). Thus, the

effect of IBMQ on the electroretinogram parameters could be due to its inhibitory effect on phosphodiesterase 6. Rolipram and cilostamide have been reported to be inhibitors of phosphodiesterase 3 (a cAMP-specific family) and phosphodiesterase 4 (a cGMP-inhibited family), respectively (Lugnier et al., 1986). In this study, we showed that these inhibitors do not have a significant effect on visual function.

Three main conclusions may be derived from the present report: (1) GF248 is a very potent inhibitor of phosphodiesterase 6. GF248 could be a useful pharmacologic tool to study the role and the regulation of phosphodiesterase 6 in visual function mainly because of the low dose required. (2) The correlation between the in vitro enzymatic IC_{50} and the efficacy to change parameters of the rat retina electroretinogram of the different compounds showed that phosphodiesterase 6 seems to be the major phosphodiesterase controlling cGMP during the light response in the retina. (3) Finally, the observation that rolipram and cilostamide have no effect on electroretinogram parameters indicates that phosphodiesterase 3 and phosphodiesterase 4 are either not or only slightly involved in light response of the retina.

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