The Influence of Phosphodiesterase Inhibitors on ERG and Optic Nerve Response of the Cat

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Reversible changes in electroretinograms (ERG) and optic nerve responses (ONR), induced by various concentrations of four different types of phosphodiesterase (PDE)-inhibitors, were recorded from arterially perfused, isolated cat eyes. In the dark-adapted eye, the implicit time of the PIII-component of the ERG, isolated by temporary hypoxia, showed a dose-dependent increase after the injection of PDE-inhibitors into the ophthalmociliary artery. The PIII-amplitude increased in response to small doses of PDE-inhibitors, whereas higher doses led to an amplitude decrease. Rod b-wave amplitude and implicit time showed similar alterations. An amplitude increase could not be observed in the rod ONR. In the light-adapted eye, 440 nm and 555 nm cones were functionally separated by means of chromatic adaptation. Their ERG and ONR implicit times were prolonged under PDE-inhibition. The amplitude behaviour of both types of cones showed a different concentration dependency. Certain concentrations of PDE-inhibitors depressed the short-wavelength sensitive cone, while, at the same time, sensitizing the long-wavelength sensitive cone. The amplitude increase of the L-cone b-wave was accompanied by a response increase of tonic ganglion cells in the ONR. Invest Ophthalmol Vis Sci 27:1395-1403, 1986.

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The function of rods. Little is known whether these findings are relevant for higher vertebrates, and even less is known about cone function. Therefore, we undertook the present study in the cat, injecting PDE inhibiting drugs into isolated, arterially perfused eyes, while recording the corneal electroretinogram (ERG) as well as the optic nerve response (ONR). Special attention was paid to the drug effects on the function of spectrally different cones, isolated by means of chromatic adaptation.

Materials and Methods

The isolated, arterially perfused cat eye preparation was used in order to avoid systemic effects of the drugs and to have controlled concentrations with intact natural blood brain barrier, free of metabolites. The eyes of 19 adult cats (2.5-3.2 kg, male and female) were enucleated under intravenous anesthesia by sodiumthiobarbiturate (5 mg/kg bw and h) and anticoagulation by heparin (1650 I.U./kg bw). Mydriasis was obtained by local administration of 5% neo-synephrine and 1% atropine eye drops before enucleation. Within 5-10 min after enucleation, the eye was perfused (2.5 ml/min, electronically monitored) with a buffered (pH = 7.4 ± 0.05) and preheated (37.5°C) mixture of Eagle's tissue culture medium and newborn calf serum, equilibrated with oxygen (pO₂ 250-300 mm Hg) via the ophthalmociliary artery, canulated with a polyethylene tube. The outer diameter of this tube was 0.5 mm and the tube tapered to a tip of about 80-100 μm. A second tube (outer diameter 0.25 mm) within the perfusion...
tube ended shortly behind the globe and was used for the separate intraarterial administration of drugs. Rate (1 ml/min) and volume of drug injection were controlled by a motor driven syringe. With the help of a screw-valve and a drop counter, perfusion flow was approximately kept constant at pre-injection values during the injection of drugs. These experiments were carried out according to the ARVO Resolution on the Use of Animals in Research.

Four different phosphodiesterase-inhibiting drugs were used: Theophylline (mw 180.17), caffeine (mw 194.19), papaverinehydrochloride (mw 375.84), and AR-L 115 BS (mw 287.34). The latter is a benzimidazole derivative with PDE inhibiting potency. Theophylline and caffeine were used for comparability of our data with the literature, as many of the referenced studies applied to one or both of these substances. Finally, papaverine was used as this drug, contrary to the other three PDE inhibitors tested, does not belong to the methylxanthines, which might lead to different effects on the visual system. Usually, the drugs were dissolved in the same oxygen saturated medium that was used for perfusion. In a few experiments, drugs were dissolved in isotonic saline after it had been checked that the short phase injection of pure saline (for 1 min) had no effect on the parameters under study.

Isolation of the PIII-component of the ERG was obtained by reducing the flow rate to approximately 1.0 ml/min and by reducing the PO2 to 100 mm Hg. These conditions were kept for about 30-40 min. Thereafter, flow rate and oxygen pressure were returned to their normal, abovementioned values. Under these conditions, the b-wave became unrecordable even in averaged responses (n = 16 or 32) and showed no tendency to recover. Stable PIII-recordings were obtained for 4-6 hr.

Two chlorided silver electrodes were used for ERG recordings, one touching the cornea, and the other touching the sclera at the posterior pole of the globe by cotton wicks moistened with saline. A second pair of electrodes allowed the recording of the optic nerve response (ONR), a sum potential of the fibers in the optic nerve. The cut end of the optic nerve was sucked into a small glass tube which contained a chlorided silver wire, embedded in saline-moistened cotton. A second chlorided silver wire, serving as reference electrode, was positioned on the optic nerve at its exit from the globe. Frequency band-pass was set at 0.1-300 Hz for both ERG and ONR recordings. After preamplification (1500X), recordings were monitored on an oscilloscope and plotted on a four channel analog recorder. Data were stored on a tape recorder for further evaluation. Computer averaging of the signals was done when recording the PIII and the cone b-wave. Only Figure 7 shows an on-line cone ERG.

Two light beams, one serving as test light and the other as adapting background, were provided by a 150 W Xenon arc lamp (Osram, West Germany). Stimulus duration and time interval between two successive test stimuli were controlled by a noncommercial electronic clock triggering an electrical shutter in the test beam. Yellow backgrounds that suppress rod and longwave-length sensitive cone activity were provided by a filter with a sharp cut-on at 515 nm (OG 515, Fa. Schott), whereas blue-green backgrounds were provided by a broad-band pass filter with a transmission of more than 20% in the range from 350-550 nm and a maximum transmission at 450 nm (BG 28, Fa. Schott). The color of the test beam was determined by 14 calibrated, narrow-band interference filters (half band-width 20 nm) ranging from 416-648 nm. A set of neutral-density filters, calibrated for each spectral composition, allowed independent variation of the irradiance of the test and adapting beam. Both beams illuminated a neutral diffuser positioned immediately in front of the eye. Irradiances and illuminances are given as corneal values and were measured by means of silicon diodes of known spectral sensitivity. Amplitudes of the PIII-component of the ERG and of the ONR were measured from the baseline of the recordings. The b-wave of the ERG was measured from the trough of the initial a-wave. Implicit times were measured from the onset of the stimulus to the maximum of the potential under consideration.

Results

The PIII was isolated in 11 eyes. A V-log I-function of the PIII was recorded before pharmacological testing. Every substance was tested at least twice in two eyes of different animals. In some eyes, two or more substances were tested in different orders so as to allow a more direct comparison of their action. The influence of PDE inhibitors on amplitude and implicit time of the isolated PIII-component of the scotopic ERG is shown in Figure 1. Measurements were made at the end of a 1-min drug injection, and compared with the pre-injection values. As the injection rate was 1 ml/min, concentrations are given in μmol/ml in order to facilitate the calculation of the absolute amount of drug injected. Wavelength (λ = 508 nm) and near threshold irradiance (E = 10^2-24 and 10^217 Quanta*s^-1*μm^-2) of the test stimulus were kept constant; a stimulus duration of 200 ms and an interstimulus interval of 1.17 s were used throughout the study. Pre-injection amplitude and implicit time were in the range of 7-10 μV and 240-250 ms, respectively. They were both standardized to 1 in the illustration. All the PIII measure-
Fig. 1. Effect of four different PDE inhibitors on the relative amplitude and implicit time of the scotopic PII component of the ERG. Note the differences in drug concentration (abscissae). AR-L 115 BS and theophylline (upper half) had a marked effect on the implicit time, whereas this effect was small in experiments with papaverine hydrochloride and caffeine (lower half). Stimulus conditions as given in the figure. In this and the following figures λ is the stimulus-wavelength and μ the background.

The amplitudes of the PII showed a biphasic dependency on drug concentration. Small concentrations led to an amplitude increase, whereas higher concentrations decreased the response. While this general pattern was the same for the four types of PDE inhibitors tested, their molar efficiency and their maximum effect differed considerably, as can be noted from the differences in the concentrations on the abscissae in Figure 1. AR-L 115 BS had the strongest and caffeine the weakest effect. The observed amplitude changes were fully reversible within 10–20 min, even with the highest concentrations tested. The effects of caffeine and papaverine hydrochloride were more prolonged than those of AR-L 115 BS and theophylline.

The implicit times of the PII showed a concentration-dependent prolongation for all the PDE inhibitors tested. However, a subdivision into two classes seems feasible. AR-L 115 BS and theophylline (upper half of Figure 1) had a very marked effect, whereas papaverine hydrochloride and caffeine caused only a small prolongation of the PII implicit time (lower half of Figure 1). As can be seen from Figure 1, alterations of amplitude and implicit time do not correlate with each other. Even a plot of AR-L 115 BS concentrations against those of theophylline that produced the same relative prolongation of implicit time showed no linear correlation. Instead, the course of an exponential function was obtained, indicating that more than one reaction is underlying the prolongation of the implicit time and that the different biochemical processes have different pharmacological affinities for the tested drugs. The time constant of the reversibility of the drug-in-
Change of rod b-wave sensitivity

![Graph showing the change of rod b-wave sensitivity](image)

Fig. 2. Concentration-dependent change in rod b-wave sensitivity (upper half) and implicit time (lower half) under the application of AR-L 115 BS.

Produced changes in implicit time was the same as for amplitude changes.

The behaviour of the rod b-wave shows interesting parallels to that of the PIII-component of the ERG. For instance, the concentration-dependent prolongation of the implicit time observed in the PIII was also found in the rod b-wave (lower half of Figure 2). This prolongation, marked with AR-L 115 BS and theophylline, less pronounced with papaverinehydrochloride and caffeine, seems to be a general feature as it could be observed in the 440 nm-cone and 555 nm-cone b-wave, as well as in the corresponding ONRs (see below). A certain drug concentration induced the same relative prolongation of implicit time in PIII, rod and cone b-wave, and in the ONRs.

The upper half of Figure 2 shows the influence of AR-L 115 BS on the sensitivity of the rod b-wave. The latter was determined as the reciprocal value of the irradiance necessary to elicit a half-maximum rod b-wave response. Low concentrations shifted the V-log I-function to the left, the half-maximum value being plotted upwards in Figure 2 as an increase in sensitivity, whereas higher concentrations decreased the b-wave sensitivity. Similar findings were obtained with the three other types of PDE inhibitors. When comparing the behaviour of rod b-wave (Fig. 2) and PIII (Fig. 1, upper left) in response to AR-L 115 BS, it should be taken into account that the concentrations underlying the b-wave changes are much smaller, as shown on the abscissa. The amplitudes of the scotopic optic nerve responses, recorded in parallel with the b-waves described above, were not increased during PDE inhibition. Even when the rod b-wave started to rise and its V-log I-function was shifted to the left, the optic nerve response decreased, thereby showing an opposite behaviour as long as low concentrations of PDE inhibitors were tested. This is exemplified in Figure 3 by some actual ERG (upper trace of each pair) and ONR (lower trace of each pair) responses recorded under the influence of theophylline.

The rod b-wave changes, as well as the ONR changes recorded in parallel, were completely or almost completely reversible within 20–30 min after the end of drug injection. The same is true for isolated cone responses discussed below. For the same concentration of PDE inhibitors, the recovery of the b-wave and optic nerve response took a longer time than did the recovery of PIII. Higher concentrations, for instance, more than...
3 μmol/ml AR-L 115 BS or more than 5 μmol/ml papaverine hydrochloride, led to an irreversible abolition of b-wave and ONR.

It is well known that in dark-adapted cats and other animals, the first flash of a pair of stimuli of high irradiance elicits a b-wave much larger than that produced by the second flash. This suppression-recovery effect has been shown to parallel rod sensitivity in the cat, and has been explained by lateral inhibitory neural processes. Consequently, the suppression-recovery effect is driven by rods. We have studied this effect under the influence of PDE inhibitors. Two stimuli (200 ms, $E = 10^{5.62}$ Quanta s$^{-1}$ s$^{-1}$) were flashed on the dark-adapted eye at an interval of 2.2 s. The time between two consecutive first pulses was at least 1 min, enough to allow complete b-wave recovery. Under these conditions, the b-wave response to the second pulse was in the range of 40–50% of that to the first pulse. In the ONR, the suppression-recovery effect is smaller, and the response to the second pulse was about 70–80% of that to the first. This may be explained by the fact that the ONR of the cat is a more cone-dominated signal than the ERG and cones do not show a suppression-recovery effect. Under the injection of PDE inhibitors, the b-wave response to the second pulse is more strongly affected than that to the first. This leads to a decrease of the ratio between the response to the second pulse (Fig. 4, inset) and the response to the first pulse as shown for different concentrations of AR-L 115 BS in Figure 4 (upper half). The corresponding amplitude ratio of the ONR exhibited smaller changes with a different time course. A weak transient decrease during the beginning of drug injection was often followed by an increase in the steady state. This is shown for theophylline (11 μmol/ml) in Figure 4 (lower half). For the drug concentrations tested, the changes in the amplitude ratio of the ONR did not exceed a range of ±10%.

In order to study the effect of PDE inhibitors on cone function, 440 nm-cones (S-cones) and 555 nm-cones (L-cones) were isolated by chromatic adaptation. The spectral sensitivity of the b-wave as determined with a constant threshold criterion during light adaptation with a yellow background (Schott OG 515) of 1420 lm/m$^2$ is represented by the filled dots in Figure 5. The solid lines in Figure 5 are Dartnall pigment nomograms with a maximum absorption at 447 nm (left) and 555 nm (right). The short-wavelength part of the spectrum was corrected for lens absorption. The luminance of the background was 5.5 log units above the rod threshold and suppressed the rod response to less than 5% of its maximum. Thus, it is obvious that, under these conditions of adaptation, stimuli near threshold irradiance will stimulate either the S- or the L-cone, when appropriate wavelengths are used.

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**Fig. 4.** (upper half) Suppression-recovery (SR) effect under the influence of different concentrations of AR-L 115 BS. The SR-effect is expressed as the ratio of the response to the second pulse to the response to the first pulse. (lower half) SR-effect in ERG and ONR under the injection of theophylline. The dashed vertical line indicates the end of drug injection.

**Fig. 5.** Spectral sensitivity of the b-wave of our preparation during light adaptation with a strong yellow background is represented by the filled circles. Open circles and arrows indicate the threshold changes induced by AR-L 115 BS (28 nmol/ml).
ternate with each other, close similarities were found of the eye. This is indicated by the open circles in Figure 5, which represent recordings under the influence of AR-L 115 BS. Comparing the breaking point in concentrations where response increase and decrease alternate with each other, close similarities were found between rod b-wave and S-cone response. For instance, for AR-L 115 BS this concentration was about 80 nmol/ml in rods and about 110 nmol/ml in S-cones, whereas concentrations of more than 800 nmol/ml were necessary to decrease the response of the L-cone.

Optic nerve responses of cat eyes elicited by S- and L-cones differ markedly. \(^1\) The L-cone ONR shows a rapid phasic and a later tonic on-effect, as well as a marked positive off-effect. The latter is absent in the S-cone, which has a predominantly tonic discharge at stimulus onset. The alterations of the S-cone ONR parallel that of the b-wave when recorded during the injection of PDE inhibitors, both being either depressed or enhanced. Contrary to this, the L-cone ONR exhibited a more complex response pattern. The phasic on-component did not show substantial changes during drug application. PDE inhibitors mainly affected the tonic on-component and the off-effect. This is shown in Figure 7. The drug concentration was the same as in Figure 6, which presents responses recorded in the steady state of AR-L 115 BS injection. Note that the amplitude does not change in the ONR recordings in Figure 7 during drug injection when it is measured immediately after stimulus onset (phasic component), while the on-response increases in its later tonic components. Note also that the amplitude of the ONR off-effect, which is clearly smaller than that of the on-effect when measured before and after drug application, surpasses the amplitude of the on-effect in the steady state of drug injection. This marked increase of the off-effect is also noticeable in the simultaneously recorded ERGs (Fig. 7, left).

**Discussion**

For the understanding of the effect of PDE inhibitors on the PIII-component of dark-adapted eyes, which is generally believed to mainly represent the electrical activity of the receptoral layer,\(^20,21\) it seems necessary to briefly summarize the role of cGMP in phototransduction, as reviewed recently.\(^1-4\) According to this concept, bleached rhodopsin activates a guanosine-binding protein. The latter activates a rod outer segment phosphodiesterase (ROS-PDE), thereby inducing a light-dependent decrease in cGMP concentration, which is high in the dark. Whether this cGMP decrease is responsible for the decrease in membrane sodium permeability, which underlies the hyperpolarizing vertebrate receptor potential, by a direct action of cGMP on the sodium channel\(^7\) or by some intermediary step, perhaps involving calcium,\(^22\) is still a matter of controversy. Besides a brief report,\(^23\) to our knowledge, physiological studies on the effect of cGMP application and PDE inhibition on phototransduction have been carried out in lower vertebrates only. Our findings on the PIII of the isolated, arterially perfused eye of the cat seem to indicate that a similar concept of phototransduction in the rods of higher vertebrates is justified. Miller and Nicol\(^6\) showed that cGMP depolarizes...
ROS of Bufo marinus. Waloga reported that a similar depolarization of single rods of the toad can be induced by the external application of PDE inhibitors. It is likely that this depolarization is responsible for the increase of the rod receptor potential observed after iontophoretic cGMP application, as well as after extracellular application of PDE inhibitors. On the other hand, Ebrey and Hood reported an amplitude decrease of rod receptor potential after the application of PDE inhibitors in the isolated, superfused frog retina. A dependency on the time of exposure to PDE inhibitors was found by Lipton et al. in the superfused toad retina. Short exposition increased the amplitude of the receptor potential, whereas long exposition decreased it. We think that these apparently contradictory findings can be explained by the concentration dependency found in our experiments (Fig. 1). Small doses of PDE inhibitors may cause a relatively weak depolarization of ROS membrane, thereby making it more sensitive to a hyperpolarizing light stimulus, whereas higher doses induce a strong depolarization that cannot be overcome by a light stimulus because the high cGMP concentration, which is created by a complete or almost complete blockage of the degrading enzyme, clamp the membrane potential on a depolarized value. According to this explanation, it was a low concentration after a short exposition and a high concentration after a long exposition that led to the results of Lipton et al. When comparing the absolute drug concentrations in our study with those applied in many of the studies quoted above, it should be kept in mind that drugs injected intravasally have to cross the blood-retinal barrier. This barrier is not present when ROS are bathed in solutions containing PDE inhibitors, or when substances are applied iontophoretically.

The fact that the relative prolongation of the implicit time was the same in potentials originating in the outer (PHI), middle (b-wave), and inner (ONR) retina points to a delay of a very fundamental process, taking place in the outer retina, probably in the ROS. We started our experiments with AR-L 115 BS and theophylline. As both caused a marked prolongation of implicit time and as this finding was in good accordance with earlier reports, we 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 prolongations in implicit time. Yet, the experiments with papaverine and caffeine refuted this hypothesis, as both evoked only a very small prolongation of implicit time. As, for instance, papaverine is known to be a stronger inhibitor of ROS-PDE than theophylline, also observed in the drug-induced changes of amplitude in our experiments, prolongation of implicit time has to be related to a process other than PDE inhibition. It has been shown that each molecule of photoexcited rhodopsin successively causes the activation of about 400 molecules of PDE, thereby providing a considerable amplification of light absorption. It also has been proposed that the phosphorylation of rhodopsin is the decisive turn-off mechanism for bleached rhodopsin. Interestingly enough, some PDE inhibitors (e.g., theophylline) inhibit the rod outer segment phosphorylation of bleached rhodopsin, whereas others (e.g., papaverine) do not. As theophylline prolongates the implicit time much more than does papaverine (Fig. 1), it seems justified to assume that the inhibition of the deactivating phosphorylation of rhodopsin is responsible for the marked prolongation in implicit time.

![Fig. 7. Simultaneously recorded ERGs and ONRs of the isolated L-cone are shown (μ = blue-green, 29 lm/m² and λ = 601 nm, D = 200 ms, E = 10^4 Quanta*s^-1*μm^-2). The b-wave increases during drug injection, whereas the baseline to maximum amplitude of the ONR does not exhibit a major change. In contrast, the tonic component of the ONR increases, accompanied by a marked increase of the off-effect. Note that, similar to observations shown in Figure 3, the drug-induced changes need some time for reaching a steady-state. All changes reported in this paper were recorded in the steady-state if not stated otherwise.](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933361/ on 10/17/2017)
observed for AR-L 115 BS and theophylline. This might also explain the dissociation of amplitude and implicit time, as visible in Figure 1. The four drugs induce similar amplitude changes by inhibiting the PDE. The implicit time is prolonged markedly by AR-L 115 BS and theophylline, mainly due to an additional effect of these drugs on rhodopsin phosphorylation, an effect not shared by papaverine and apparently also not shared by caffeine, which have only a slight effect on the implicit time. In conclusion, the dissociation of amplitude and implicit time may be caused by different enzymatic targets of the drugs.

Interpretation of the b-wave and ONR recordings is more difficult, as neural elements in the middle and inner retina which also contain cyclic nucleotides (although considerably less than ROS) generate these potentials. Different pharmacological affinities of these systems to the applied drugs might explain, for instance, the opposite behaviour of rod b-wave and corresponding ONR. Yet, it should be pointed out that the increase of the receptor potential (PIII) recorded under the influence of PDE inhibitors seems to be transmitted to those cells generating the b-wave, as the latter also increases, although it is of opposite electrical polarity. Thus, it may be speculated that the changes observed in the cone b-waves reflect similar changes in cone receptor potential. The similarity of rod and S-cone behaviour on the one hand, and the large difference in concentration between equally effective PDE inhibitor doses for S- and L-cone amplitude on the other, indicate similarities in cyclic nucleotide metabolism between rods and S-cone and differences between S- and L-cones. This is in agreement with the hypothesis that, in cones, cyclic AMP may serve a role comparable to that of cyclic GMP in rods.\textsuperscript{32,33} As this hypothesis is based on biochemical findings in homogenized retinae of animals rich in cones, it does not exclude that, in a single cone type (e.g., S-cone), cGMP dominates.

Similarities in the cyclic nucleotide metabolism of S-cone and rod might also help to understand the clinical observation that rod degenerations in the retina are often accompanied by an early loss of sensitivity in the blue range of the spectrum.\textsuperscript{34} Similarities between rods and S-cones are also observed in their electrical response behaviour.\textsuperscript{10} The higher doses of PDE inhibitors necessary in L-cones are also in agreement with the observation\textsuperscript{27} that application of PDE inhibitors causes a much greater rise in cGMP than in cAMP, at least in rod outer segments. It has been proposed\textsuperscript{25,36} that phasic ganglion cells mainly encode luminance, whereas tonic ganglion cells mediate color. Assuming that in the ONR phasic ganglion cells are responsible for the transient response at stimulus onset and that the more sustained on-response in the ONR bases on the activity of tonic ganglion cells, the drug-induced changes in the L-cone ONR might mean an increase of the output of the chromaticity channel, while keeping the luminance channel constant.

The high susceptibility of rods and S-cones in contrast to L-cones observed in our electrophysiological recordings can be detected psychophysically and electrophysiologically in man as well. Zrenner et al\textsuperscript{13} reported a dose-dependent, reversible disturbance of S-cone and rod sensitivity in humans who had received an intravenous injection of AR-L 115 BS, while L-cones were not affected by the dosage applied. PDE inhibitors do not only change the action of single cells, but also seem to have an effect on cellular interaction. Measuring transient tritanopia, Zrenner\textsuperscript{14} described a disturbance of cone interaction in humans who had received PDE inhibitors. Our findings of drug-induced changes in the suppression-recovery effect, that is believed to base on rod-driven lateral inhibitory neural processes in the retina, point to an effect of these drugs on rod interaction as well.

**Key words:** phosphodiesterase inhibition, methylxanthines, electroretinogram, optic nerve response, rod function, cone function

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