Theophylline Abolishes the Light Peak in Perfused Cat Eyes

Theophylline, a phosphodiesterase inhibitor, was applied to the arterially perfused cat eye in vitro. At a concentration of 850 μM, theophylline abolished the light peak of the DC-ERG and led to a 20% increase in perfusate flow to the eye. The effects were reversible. An experimentally-induced increase in flow per se by 20% resulted in a slight enhancement of the light peak. The data suggest the involvement of cyclic nucleotides in the generation of the light peak. Theophylline also caused a decrease in the standing potential of the eye, an increase in the b-wave amplitude, and a decrease in the c-wave amplitude in a dose-dependent and reversible manner. Invest Ophthalmol Vis Sci 28:700-706, 1987

To test the hypothesis that cAMP is involved in the generation of the light peak, the methylxanthine theophylline was used. One of the actions of methylxan-thines is to inhibit phosphodiesterases that hydrolyze cyclic nucleotides. This action results in an increase in the intracellular concentrations of cyclic nucleotides. In this framework, theophylline was expected to produce effects similar to those seen with dibutyryl cAMP. Theophylline did abolish the light peak, but this effect was preceded by a decrease, rather than an increase, in the standing potential. This finding is apparently at odds with an allosteric role of cAMP in the generation of the light peak. Although dibutyryl cAMP and theophylline affected the standing potential differently, they both reduced the light peak. It is possible that this reduction is mediated by cAMP. 

To characterize the effect of theophylline more fully, the marked effects on the flow and electroretinogram (ERG) are also described.

Materials and Methods

This study adhered to the ARVO Resolution on the Use of Animals in Research. Details of the isolated, arterially-perfused cat eye technique have been published. The experimental protocol was identical to one described previously. Therefore, only a brief description of the procedure is given here.

Eyes were enucleated from cats under sodium pentobarbital anesthesia, occasionally supplemented with surital. The ophthalmic arteries were cannulated, and the eyes perfused with tissue culture medium supplemented with newborn bovine serum, which was either buffered with HEPES and bubbled with 100% O₂ or buffered with bicarbonate and bubbled with 5% CO₂/95% O₂. Temperature was maintained at 37°C, and

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just before it flowed into the eye. By varying the injection rate, various concentrations of theophylline (0.25 to 2 mM) could be delivered. Theophylline was obtained from Sigma Chemical Co., St. Louis, MO.

## Results

### The Effect of Theophylline on the Standing Potential and Light Peak

Figure 1 illustrates the effect of 850 μM theophylline on the maintained and light-evoked electrical activity of the isolated, arterially-perfused cat eye. The top, middle, and bottom traces in Figure 1 are consecutive segments of a continuous time recording of the voltage potential measured between the vitreous and the posterior pole of the eye. The hatched horizontal bars signal the occurrences of a 1-min white light pulse (70 cd/m²). For point of reference, we define time zero (t = 0 min) to correspond to the onset of the first white light pulse. This light pulse evoked an ERG of which only the c-wave (the large “spike” in the recording) can be resolved, due to the slow dynamics of the pen recording. On termination of the light pulse there was a small off-response followed by the “second c-wave” (the peak occurring about 2 min after light onset). Approximately 5.5 min after light onset, the voltage attains a maximum which is called the light peak. The potential then swings slowly in the negative direction. Superimposed upon this negative swing is a sequence of ERGs evoked by 20 msec red light flashes presented every 30 sec beginning at t = 14 min. At t = 22 min, approximately 850 μM theophylline was arterially delivered to the eye. The theophylline caused the standing potential to decrease and produced changes in the ERG, which will be described in a later section. The standing potential slowly rebounded and approached a new plateau. The red light flashes were discontinued, and a second white light pulse was presented at t = 40.5 min. The response is shown in the middle trace of Figure 1. The c-wave and second c-wave of this response were enhanced; the light peak was virtually abolished. The red light flashes were resumed. At t = 54 min, the theophylline injection was terminated; this resulted in a large increase of the standing potential. The standing potential gradually declined to a new plateau. When the potential reached a plateau, the red light flashes were discontinued, and a third white light pulse was presented. The response to the third (recovery) white light pulse was similar to that evoked by the first (control) pulse. The reduction of the light peak with theophylline was confirmed in other trials. In six out of seven trials, theophylline (ranging from 0.5 mM to almost 2 mM) clearly reduced the light peak (from 72% to 7% control amplitude); the outcome of the remaining trial was unclear because the baseline was drifting upwards rapidly during the test light peak.

As indicated in legend of Figure 1, the theophylline injection caused a 20% increase in perfusate flow rate. Since the perfusion pressure was constant, the increase in flow indicated that the vascular resistance of the eye had decreased. The question arises: could the decrease
The Effect of an Increase in Flow on the Standing Potential and Light Peak

To estimate the contribution of flow to the effects shown in Figure 1, the flow to the eye was increased by 20% by readjusting a flow valve in the perfusion system. The results are shown in Figure 2. The response to a 1-min diffuse white light shown at the bottom of Figure 1 is reproduced at the top of Figure 2. A period during which the eye received a 20% increase in flow is shown in the middle trace of Figure 2. After the standing potential had risen from a negative swing, the flow rate to the eye was manually increased at t = 113.5 min (with time zero still corresponding to the onset of the first white light pulse in Fig. 1). In agreement with a previous study, the change in flow hardly affected the standing potential. The effect on the ERG will be described later. A white light pulse was presented at t = 123 min. The evoked light peak, shown in the middle trace of Figure 2, was slightly larger than control. The perfusate flow was then adjusted to the control level, and a recovery light peak was evoked by a white light pulse at t = 157 min. The recovery light peak, shown at the bottom of Figure 2, was similar to the control light peak, shown at the top of Figure 2.

It seems unlikely that the reduction of the light peak with 850 μM theophylline was due to the concomitant increase in flow. Figure 3 shows the two test light peaks from Figures 1 and 2. The solid curve is the response obtained during 850 μM theophylline and the drug-induced increase in flow; the dotted curve is the response obtained during a manually-induced increase in flow without theophylline. The responses were obtained in the same eye and under the same total perfusate flow. Unless the drug-induced and manually-induced increases in flow resulted in markedly different distributions of flow within the eye, the reduction of the light peak under theophylline could not have been caused by the concomitant increase in flow.

The Effect of Theophylline on the ERG and Flow

Figure 4 illustrates the effects of approximately 2 mM theophylline on the ERG. The three recordings were plotted with an oscillographic recorder, which had faster dynamics than the pen recorder used for plotting Figures 1–3. The ERGs were evoked with the standard 20-msec diffuse red light flash. The control ERG was obtained before the injection of theophylline. The a-
wave was slight, the b-wave amplitude measured from the a-wave was almost 600 μV, and the c-wave amplitude measured from the baseline was approximately 250 μV. After 9.5 min of injecting theophylline, the ERG in the middle of Figure 4 was obtained. The a-wave virtually disappeared, the b-wave increased in amplitude to about 700 μV, and the c-wave reversed its polarity. The theophylline injection was terminated, and after 21.5 min of recovery the ERG on the right-hand side of Figure 4 was obtained. The ERG was only partially recovered at this time. After 50 min of recovery, the b-wave amplitude, c-wave amplitude, and flow recovered to 98.9%, 91.9% and 98.3% of the control values, respectively. (To demonstrate the reversal of the c-wave, concentrations exceeding 1 mM theophylline were required; it is remarkable that after exposure to these high concentrations, which cause striking changes in the ERG and light peak, the electrical responses of the perfused cat eye recovered.)

Since theophylline induces an increase in perfusate flow, some of the effects shown in Figure 4 may be flow-related. As shown in Figure 5, the increase in flow helps to increase the b-wave amplitude, but not to reverse the c-wave. Figure 5 is a comparison of two experimental series in the same eye. The solid lines are results obtained when 1 mM theophylline was injected; the dotted lines are results obtained when the flow was manually increased by about 17.5% in the absence of theophylline. The standard red light flash was presented every 30 sec during the two series. The b- and c-wave

![Fig. 4. The effect of 2 mM theophylline on the ERG. The control ERG was obtained prior to the injection of theophylline. The theophylline ERG was obtained after theophylline had been continuously injected for 9.5 min. The recovery ERG was obtained 21.5 min after the theophylline injection was terminated. Theophylline enhanced the b-wave, which was slow to recover. A striking effect of theophylline was the "reversal" of the c-wave, an effect which was readily reversible. Note in Figure 1 that the reversal of the c-wave was overcome when a 1-min pulse of diffuse white light was used to evoke the light peak (left part of second segment). In the above ERG experiment, the control perfusate flow was 1.30 ml/min; the theophylline perfusate flow was 1.47 ml/min; and the recovery perfusate flow was 1.40 ml/min. Theophylline (40 mM stock solution) was injected at a rate of 0.0696 ml/min which yields an arterial concentration of approximately 2 mM at the beginning of the injection period, and 1.8 mM by the time of the theophylline ERG (the dilution with time was caused by the gradual increase in perfusate flow induced by theophylline). In all cases the photic stimulus was a 20 msec diffuse red (620 nm) light flash (250 cd/m²).

![Fig. 5. Time course of effect of 1 mM theophylline (solid curves) and a 17.5% manual flow increase (dotted curves) on the b-wave amplitude, c-wave amplitude, standing potential, and perfusate flow. The theophylline and flow experiments were conducted in the same isolated, perfused cat eye. ERGs were evoked every 30 sec with a 20-msec diffuse red (620 nm) light flash (250 cd/m²). The amplitude of the b-wave was measured from the a-wave trough to the b-wave peak; the amplitude of the c-wave was measured from the baseline to the peak of the c-wave or, in the case of reversed polarity during theophylline, to the trough of the potential change which had the same implicit time as the c-wave. Values for b-wave amplitude, c-wave amplitude, and flow are expressed as percent of control values. Control values were obtained by averaging three measurements prior to theophylline-induced or flow-induced changes. The control value for the standing potential was redefined to be zero mV. The standing potential and flow were measured just prior to each ERG. Time zero was arbitrarily defined to correspond to the time at which theophylline effects first seemed to occur; the onset of theophylline injection began at t = −0.64 min, the delay in the onset of effects is in part due to dead volume from the site of injection to the eye. The dotted curves from the flow experiment were positioned by aligning the flow curves in the bottom right panel, so that they overlapped during the initial stage of the respective flow changes. As the flow curves show, the perfusate flow was manually increased for the flow experiment in such a way as to approximate the change in flow induced by the 1-mM theophylline injection. The manual flow increase caused a considerable increase in b-wave amplitude, and slight increases in the c-wave amplitude and standing potential. The 1 mM theophylline comparison caused a larger increment in b-wave amplitude, and very large decreases in the c-wave amplitude and standing potential. Although local variations in flow distribution within the eye can not be discounted, it seems unlikely that the decreases in the c-wave amplitude and standing potential during theophylline injection were caused by the increase in perfusate flow. On the other hand, part of the b-wave amplitude increase during theophylline injection could be due to the increased flow.)

![Fig. 6. The ERG in the middle of Figure 4 was obtained. The a-wave amplitude, c-wave amplitude, standing potential, and perfusate flow are expressed as percent of control values. Control values were obtained by averaging three measurements prior to theophylline-induced or flow-induced changes. The control value for the standing potential was redefined to be zero mV. The standing potential and flow were measured just prior to each ERG. Time zero was arbitrarily defined to correspond to the time at which theophylline effects first seemed to occur; the onset of theophylline injection began at t = −0.64 min, the delay in the onset of effects is in part due to dead volume from the site of injection to the eye. The dotted curves from the flow experiment were positioned by aligning the flow curves in the bottom right panel, so that they overlapped during the initial stage of the respective flow changes. As the flow curves show, the perfusate flow was manually increased for the flow experiment in such a way as to approximate the change in flow induced by the 1-mM theophylline injection. The manual flow increase caused a considerable increase in b-wave amplitude, and slight increases in the c-wave amplitude and standing potential. The 1 mM theophylline comparison caused a larger increment in b-wave amplitude, and very large decreases in the c-wave amplitude and standing potential. Although local variations in flow distribution within the eye can not be discounted, it seems unlikely that the decreases in the c-wave amplitude and standing potential during theophylline injection were caused by the increase in perfusate flow. On the other hand, part of the b-wave amplitude increase during theophylline injection could be due to the increased flow.)

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amplitudes of each ERG were measured and plotted on the left-hand side of Figure 5. Zero time corresponds to the time of occurrence of the first ERG that exhibited a change produced by the test condition. In addition, the changes in standing potential and flow at times corresponding to the occurrence of the ERGs are plotted on the right-hand side of Figure 5. As Figure 5 shows, the manual increase in flow was made to mimic the theophylline-induced flow increase; however, it is not known whether the distribution of flow within the eye was the same under the two test conditions. Confirming earlier reports, the manual increase in flow resulted in an increase in the b-wave amplitude and a slight increase in the standing potential. The effect of changes in flow on the amplitude of the c-wave has been reported to be variable. In the case shown in Figure 5, the manual flow increase caused the c-wave amplitude to increase. In contrast, theophylline caused large decreases in the c-wave amplitude and standing potential. The effect of theophylline on the standing potential illustrated in Figure 5 is in agreement with similar observations made in a preceding section.

The effects of theophylline on the ERG, standing potential, and flow are graded with concentration. This is illustrated in Figure 6, which contains the results of injecting 0.25, 0.5, 1, and 2 mM theophylline into the same eye. Note that at 2 mM theophylline there are apparent depressions in the increases in flow and b-wave amplitude. Also, while there is an orderly progression of the magnitude of effect with onset of theophylline, the same is not true at termination of theophylline. The concentration dependence seen in Figure 6 is summarized by the circles (and solid lines) in Figure 7. The circles represent measurements made after 6 min of theophylline (two series in addition to the four shown in Figure 6 are included). The squares (and dashed lines) are data obtained from a second eye. Although the flow increases were not as pronounced in the second eye as in the first, the effects of theophylline on the b- and c-wave amplitudes and the standing potential were similar in the two eyes.

Discussion

The results reported here demonstrate that arterially-injected theophylline affects light-evoked responses of the retinal pigment epithelium. Phosphodiesterase inhibitors have been used previously in the retina to study the role of cGMP in phototransduction. In these studies, phosphodiesterase inhibitors depolarized the membrane potential and temporarily enhanced the photoresponse of rod photoreceptors. Such an action would be expected to cause an increase in standing potential and an increase in a-wave amplitude in our preparation. As shown in Figures 1, 5, 6, and 7, the standing potential decreased instead of increased when theophylline was injected. Furthermore, in one preparation, an isolated PI3 response was recorded, most likely due to inadequate retinal perfusion, and when 0.5 mM of theophylline was injected, the PI3 remained constant in amplitude (instead of increasing), while the standing potential decreased. It should be noted that, in the perfused cat eye, 1 mM IBMX affects photoreceptor activity in the expected manner. However,
IBMX is 40 times more potent than theophylline in affecting toad rod activity, so apparently the maximum dose of 2 mM theophylline used in the current study was below the threshold level needed to affect photoreceptors in the isolated, arterially-perfused cat eye. For these reasons, it seems likely that the effects reported in this paper were due to an action of theophylline on cells other than photoreceptors.

The retinal pigment epithelium is likely one of the sites of the theophylline action reported here. The basal membrane of the pigment epithelium is exposed to the theophylline present in the choroidal circulation. It would be reasonable to expect theophylline to affect retinal pigment epithelium before affecting the photoreceptors. We found that theophylline greatly suppressed the light peak response, which is generated by a depolarization of the basal membrane of the retinal pigment epithelium. In addition, if the lowering of standing potential that we observed with theophylline is due to a hyperpolarization of the basal membrane of the pigment epithelium, the accompanying decrease in the amplitude of the c-wave would fit the correlation between basal membrane potential and c-wave amplitude seen under many other conditions. For these reasons, the basal membrane of the retinal pigment epithelium is a likely site of action of theophylline.

Another probable site of action of theophylline is arteriolar muscle, since theophylline increased the flow of perfusate to the eye. As shown in Figure 5, the increase in flow may account for part of the increase in b-wave amplitude seen with theophylline. It is unknown whether the remaining increase in b-wave amplitude is a result of action on the basal membrane of the pigment epithelium, or whether there are additional sites, such as glial cells, at which theophylline acts.

Although probable sites at which theophylline acts can be suggested, the mode of action is not known. As mentioned previously, theophylline was used to test the possibility that cAMP is involved in the generation of the light peak. The idea is that an increase in the cAMP level in the retinal pigment epithelium activates a protein kinase, which in turn phosphorylates proteins, causing the basal membrane to depolarize. Theophylline, acting as a phosphodiesterase inhibitor, can raise the intracellular level of cAMP. The results do not support this idea of a cAMP-generated light peak, because theophylline resulted in a decrease in standing potential. However, it is unknown whether theophylline actually increased the cAMP level in the retinal pigment epithelium, so the results do not refute the idea. Additional biochemical studies, including measurements of cAMP levels, are required. Biochemical studies are needed to determine whether the present theophylline effects were produced by increases in cAMP levels, or by other known actions of theophylline: inhibition of adenylate cyclase, release of Ca++, binding with adenosine receptors, or modification of proton gradients. Previously, we have observed cAMP, Ca++, and acid-base balance to affect the electrical activity of the retinal pigment epithelium. Dibutryl cAMP can depress the light peak, but this is preceded by an increase in the standing potential. Ca++ can also block the light peak, but this occurs without any effect on the standing potential. Certain changes in acid-base balance also depress the light peak and are accompanied by predictable changes in b-wave amplitude, c-wave amplitude, and standing potential.

The current study characterized the effect of theophylline on the ERG and light peak of the isolated, arterially-perfused cat eye. It is clear from the results that theophylline can influence the light-evoked responses of the retinal pigment epithelium. Further electrophysiological and biochemical studies are needed to elucidate the precise nature of the theophylline action on the pigment epithelium. Such studies may eventually lead to a better understanding of the mechanisms underlying the light-evoked responses of the retinal pigment epithelium, and provide us with information about their function.

Key words: light peak, electrooculogram, electroretinogram, theophylline, cAMP, retinal pigment epithelium

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References


