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THE EFFECT OF PHOSPHODIESTERASE INHIBITORS ON THE ELECTRICAL ACTIVITY OF TOAD RODS

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SUMMARY

1. The membrane potential of toad rods was recorded during addition of small amounts of phosphodiesterase inhibitors to the extracellular medium.

2. Separate application of 3-isobutyl-1-methylxanthine (IBMX), caffeine, theophylline, papaverine and RO 20–1724 slowed down the time course of rod photoresponse to dim flashes of light. These changes were associated with a two to six-fold increase in the amplitude of photoresponse. The effects on kinetics may be described simply by an expansion of the photoresponse time scale.

3. When the drug concentration was raised above a certain level, the rods showed *supralinear* behaviour whereby doubling of the intensity of a dim flash could increase the response more than two-fold. Under similar conditions rods also showed *light sensitization* whereby responses to dim flashes were enhanced in the presence of dim backgrounds.

4. Taking the drug concentration that induced a two-fold increase in the time-to-peak, IBMX was found the most effective compound, followed by papaverine, RO 20–1724, theophylline and caffeine with relative effectivities 1, 1/2, 1/7, 1/40 and 1/100.

5. Sensitivity, kinetics and supralinear behaviour may be restored to normal by steady background illumination while still in the presence of IBMX. However the intensity of the steady light, needed to restore the sensitivity to control levels, is not sufficient to accelerate the kinetics back to control values.

6. In the presence of 50 μM-IBMX a dim steady background of light enhanced the response to dim flashes. When the intensity of the light background was increased rods were desensitized and the supralinear behaviour disappeared.

7. The antagonism between the effects of IBMX and the effects of background illumination on the kinetics of photoresponse suggests that phosphodiesterase activity controls the time course of light response in vertebrate rods.

INTRODUCTION

The process of phototransduction in vertebrate rods begins with the photoisomerization of rhodopsin molecules (Wald, 1968) and terminates with a decrease in the Na^+ current that flows across the membrane of the outer segment in darkness

(Hagins, Penn & Yoshikami, 1970). Knowledge of the intermediate steps is still incomplete. Hagins (1972) suggested that a light-induced increase in Ca^{2+} concentration closed ionic channels. Other studies suggest the involvement of cyclic nucleotides (see Hubell & Bownds, 1979). It is known that the concentration of guanosine-3′,5′cyclic-monophosphate (cyclic GMP) in rods is modulated by light mainly through the activation of a guanosine-5′-triphosphate (GTP)-dependent phosphodiesterase (Miki, Keirns, Marcus, Freeman & Bitensky, 1973; Chader, Herz & Fletcher, 1974; Goridis & Virmaux, 1974; Woodruff & Bownds, 1979; Bignetti, Cavaggioni & Sorbi, 1978). An essential step in this process is the activation of an intermediary protein which in turn activates phosphodiesterase even in the absence of photolysed rhodopsin (Fung & Stryer, 1980). Since the light-modulated currents must reflect the critical processes regulated by light within the rod, it is perhaps interesting to study the effects of modifying the activity of phosphodiesterase on the electrical responses of rods in detail. In this paper we analyse the effects of compounds known to inhibit the hydrolytic activity of phosphodiesterase on rod photoresponses. We shall show that the response of rods treated with phosphodiesterase inhibitors is drastically altered both as far as sensitivity and kinetics are concerned. All these effects may be antagonized by steady illumination, thus suggesting that cyclic nucleotide metabolism controls some of the essential features of rod photoresponse. Some of these results have already been presented in summary form (Capovilla, Cervetto & Torre 1982*a*, *b*).

METHODS

Experiments were performed on isolated perfused retina of *Bufo bufo*. Preparation, perfusion, recording techniques, light stimulation and data analysis have already been described (Capovilla, Cervetto & Torre, 1980).

Solutions. 3′-isobutyl-1-methylxanthine (IBMX) (Sigma) was dissolved in Ringer solution by heating and gentle stirring for about 30 min. Caffeine and theophylline (Sigma) were dissolved in Ringer solution at room temperature by stirring. RO 20–1724 and Diazepam (Roche), were dissolved using the same procedure as used for IBMX. Chlorpromazine (Sigma) and trifluoperazine (Maggioni) were first dissolved in an acidic medium and the pH was then gradually increased to 7·6 by adding NaOH. Papaverine (Sigma) was dissolved in a Ringer solution buffered at pH 7·6 with Tris (Sigma). The composition of the normal Ringer solution was (mM): NaCl, 110; KCl, 2·6; $CaCl_2$ 1·6;$MgCl_2$, 1·6; HCO_3^-, 22; glucose, 10.

RESULTS

The action of IBMX on the electrical activity of rods

When micromolar amounts of IBMX were added to the perfusate, substantial changes in the electrical activity of rods were observed within a few seconds. Fig. 1 illustrates the effects of 20 μM-IBMX on both membrane potential and voltage response of a rod. In this example the intensity of the test flash was equivalent to 6800 photoisomerizations (Rh*)/rod. Shortly after the application of the test solution, the resting potential decreased from the control value, of about -40 mV, and became stabilized at the new value of -34 mV within 2 min. Concomitantly, the amplitude of the photoresponse was increased and the time course prolonged. It was also noticed that, once stabilized, these effects were maintained as long as the rod was

exposed to the drug and that they were completely reversible upon washing with control Ringer solution. The effects of IBMX on the resting potential of rods were complex and varied in different experiments. It was noticed that when the drug was used at concentrations below 20 μM, rods were transiently depolarizing by a few millivolts. Then, within 2 min, the membrane potential nearly returned to the control level and the photoresponse increased, becoming stabilized at values significantly higher than the control values. Usually, the application of 50 μM-IBMX was followed by maintained depolarization of the rod membrane (17 mV on average). The voltage response to a bright flash progressively decreased in the presence of millimolar concentrations of IBMX and was eventually abolished.

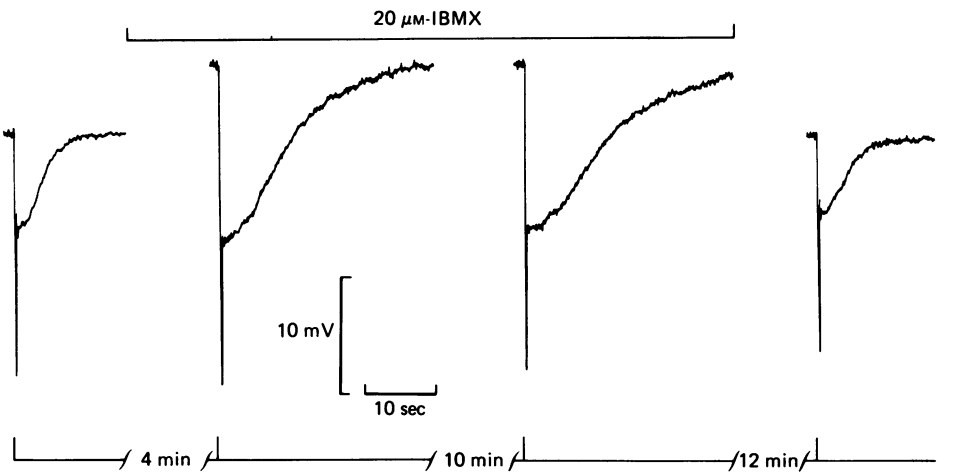

Fig. 1. Effect of 20 μM-IBMX on the electrical activity of a rod. 20 μM-IBMX was present in the perfusate during the time indicated by bars at the top. Dark resting potential in control Ringer solution was −40 mV. Bottom trace is light monitor. Monochromatic light of 510 nm; flash duration 20 msec. The flash intensity was equivalent to 6800 photoisomerizations (Rh*)/rod.

Fig. 2 shows the effects of 20 (*B*), 50 (*C*) and 100 (*D*) μM-IBMX on voltage responses to brief flashes of light of increasing intensity. The dark membrane potential depolarized by 8, 12 and 14 mV in the three test solutions. Another remarkable effect of IBMX was that the time course of photoresponses increased with drug concentration. The time-to-peak (t_{pk}) of responses to dim flashes of light increased from about 0·7 sec (*A*), to 3 sec in the presence of 20 μM-IBMX (*B*), to 4·7 sec in 50 μM-IBMX (*C*) and to 6·8 sec in 100 μM-IBMX (*D*). It was noticed that in the presence of IBMX concentrations above 20 μM, the usual relation between stimulus intensity and response amplitude was modified i.e. by increasing the stimulus strength by n times the response increased more than n times.

Kinetic changes

A simple and effective way of expressing the changes that IBMX induces in the light response was to measure the time-to-peak of response to dim flashes of light. The time-to-peak of response to dim flashes in the linear range was plotted against

the concentration of IBMX in Fig. 3 for fifteen rods. From 5 to 30 μM-IBMX, the t_{pk} increased almost linearly from 0·65 to 5·3 sec. When the drug concentration was increased above 50 μM, the t_{pk} further increased and in the presence of 500 μM-IBMX it was prolonged by thirty times with respect to control values (Fig. 3*B*).

With larger amounts of IBMX, the time course of the photoresponse could last for minutes, but these effects were difficult to analyse because under similar conditions responses progressively disappeared. The changes in photoresponse kinetics observed

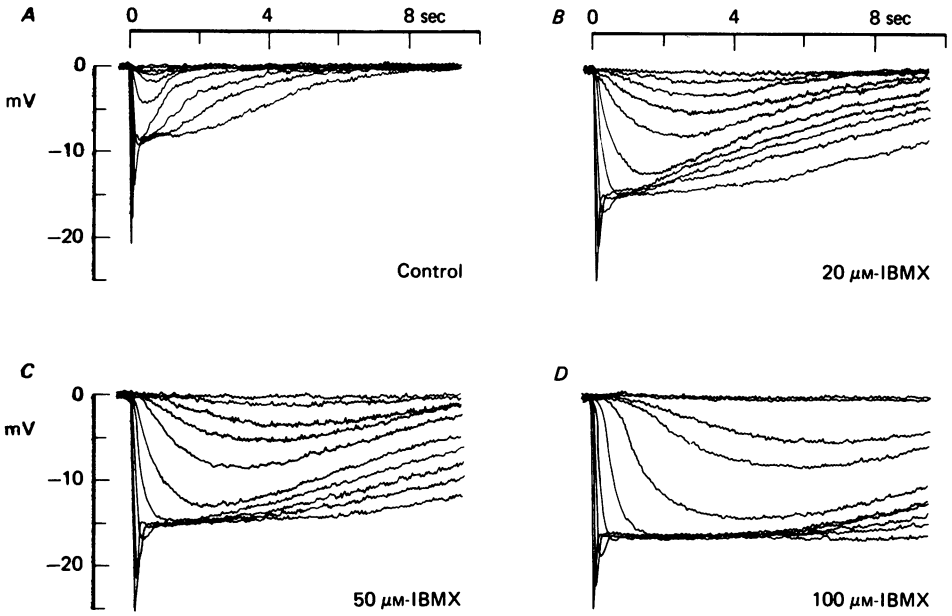

Fig. 2. Effect of 20 μM-IBMX (*B*), 50 μM-IBMX (*C*) and 100 μM-IBMX (*D*) on the electrical activity of a rod. Dark resting potential in control Ringer solution (*A*) was −43 mV, in *B* was −35 mV, in *C* was −31 mV and in *D* was −29 mV. Monochromatic light of 510 nm, flash duration was 20 msec the onset of which was coincident with the origin of time scale. The flash intensities were equivalent to 0·4, 0·7, 1·5, 2·3, 5, 21, 96, 320, 1400, 5300 Rh*/rod in *A*, *B*, *C* and *D*.

in the presence of low concentrations of IBMX (up to 20 μM) may be explained simply by an expansion of the time scale of photoresponse as shown in Fig. 4. In *A*, the voltage response of a rod to a flash of light, equivalent to 1·3 Rh*/rod is shown in both control conditions and in the presence of four different IBMX concentrations (5, 10, 20, and 30 μM). It was noticed that both amplitude and t_{pk} of voltage response increased in relation to the concentration of IBMX. Fig. 4*B* shows that when the responses obtained in 5, 10 and 20 μM-IBMX (thickly traced records) are scaled to the same peak amplitude and t_{pk} of control responses (thinly traced records) they almost coincide over the entire time course.

Kinetic studies, first conducted on *Limulus* visual cells (Fuortes & Hodgkin, 1964), showed that light responses could be described as a sequence of linear transformations equivalent to a chain of low-pass filters. The same procedure was later applied to photoresponse in rat rods (Penn & Hagins, 1972), turtle cones (Baylor, Hodgkin &

Lamb, 1974) and toad rods (Cervetto, Pasino & Torre, 1977; Baylor, Matthews & Yau, 1980). A plausible physical meaning of this model might be a chain of chemical reactions initiated by absorption of a photon by a chromophore:

$$h\nu \rightarrow Y_1 \xrightarrow{\alpha_1} Y_2 \xrightarrow{\alpha_2} \ldots Y_n \xrightarrow{\alpha_n}, \quad (1)$$

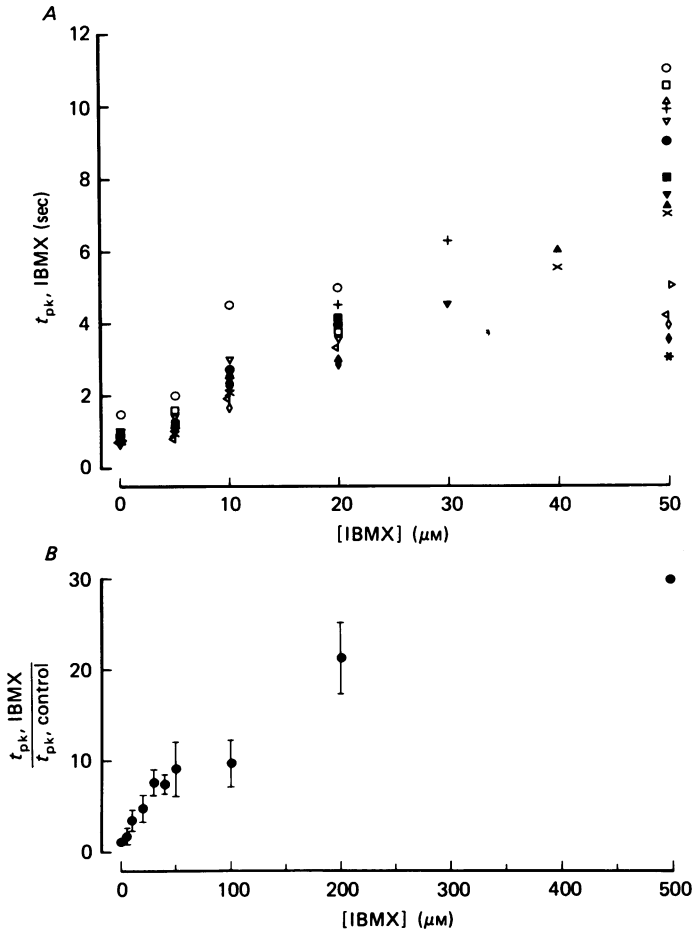

Fig. 3. Dependence of time-to-peak (t_{pk}) of dim flash voltage response on extracellular IBMX. *A*, time-to-peak of photoresponse for fifteen different rods against extracellular IBMX. *B*, average time-to-peak and standard deviation against concentration of extracellular IBMX. Collected data from thirty-seven cells. For IBMX concentration below 20 μM, the time-to-peak was measured in the linear range. For IBMX concentration above 20 μM the time-to-peak was measured in voltage responses, the amplitude of which was below 3 mV.

where $\alpha_1, \alpha_2 \ldots \alpha_n$ are rate constants and $Y_1, Y_2 \ldots Y_n$ are products of the reactions. It is assumed that the time course of the photocurrent follows the kinetics of Y_n. When scheme (1) is used to explain the time course of rod photoresponses (Cervetto *et al.* 1977; Baylor *et al.* 1980) the existence of at least four to six 'slow stages' ($\alpha_i > 100$/sec) is implied. The physical identity of these stages is not known nor is

it clear whether they reflect the build up of an internal substance capable of modifying ion channel permeability or transitions in the state of the light-sensitive channels. The observation that small concentrations of IBMX affect the kinetics of rod response, as if there was an expansion of the time scale, may offer a clue to the understanding of the nature of the 'slow stages'. Adopting scheme (1), the effects on

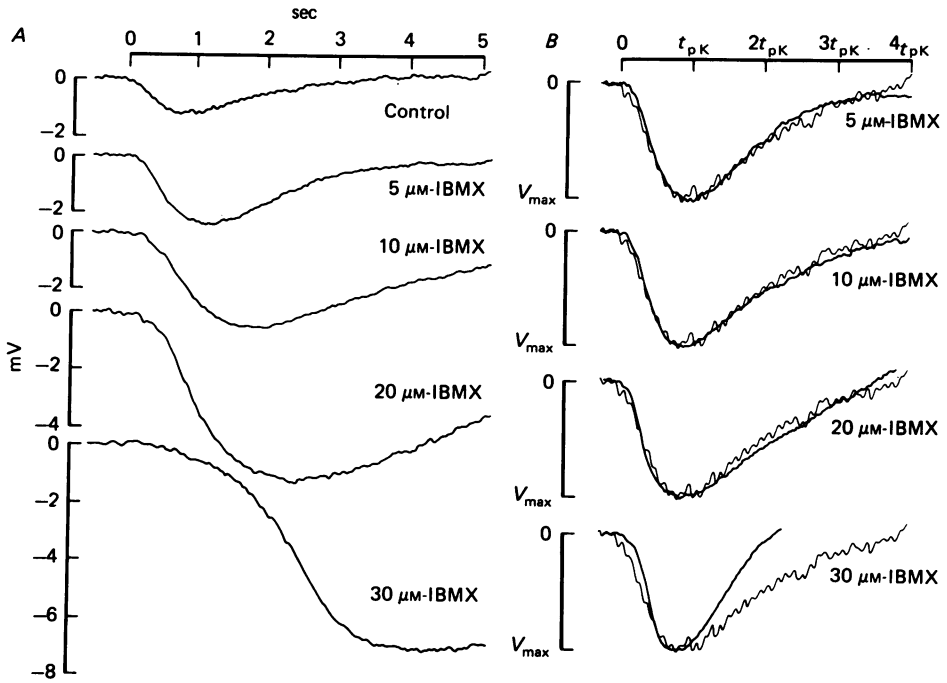

Fig. 4. Effect of different concentrations of IBMX on the voltage photoresponse of a rod to a light flash equivalent to 1·3 Rh*/rod. *A*, photoresponse in control solution and in the presence of 5, 10, 20 and 30 μM-IBMX. Each trace is the average of eight responses. The dark membrane potential was −45 mV in control conditions and −44, −42, −40 and −38 mV in the presence of 5, 10, 20 and 30 μM-IBMX, respectively. *B*, voltage response recorded in the presence of IBMX (thick trace) scaled to the same time-to-peak t_{pk} and amplitude as the control voltage response (thin trace). Monochromatic light of 510 nm, flash duration of 20 msec, the onset of which was coincident with origin of time scale.

the kinetics of photoresponses observed with small amounts of IBMX may be reproduced by decreasing *all* the rate constants by the same factor. Accordingly 5 μM-IBMX in the external medium would halve the values of α_i.

Effects of IBMX on stimulus–response characteristics

As reported earlier (Capovilla *et al.* 1982*a*, *b*) when the concentration of IBMX was raised above 20 μM, *supralinear* behaviour of the rods was detected. This phenomenon was found to depend on the IBMX concentration rather than on membrane potential.

Fig. 5*A* illustrates voltage response to flashes of increasing intensity (from 7·9 to 648 Rh*/rod) in the presence of 50 μM-IBMX. The relation between light intensity (I) and amplitude of response (ΔV), evaluated at different times from the onset of

light, did not change appreciably during the initial 5 sec. The experimental points measured at 0·5, 0·75, 1, 1·5 and 3 sec after the onset of light fit the same curve when shifted along the abscissa. The dashed line drawn through the experimental points in Fig. 5*B* is:

$$\Delta V = I + 0{\cdot}1\, I^3, \tag{2}$$

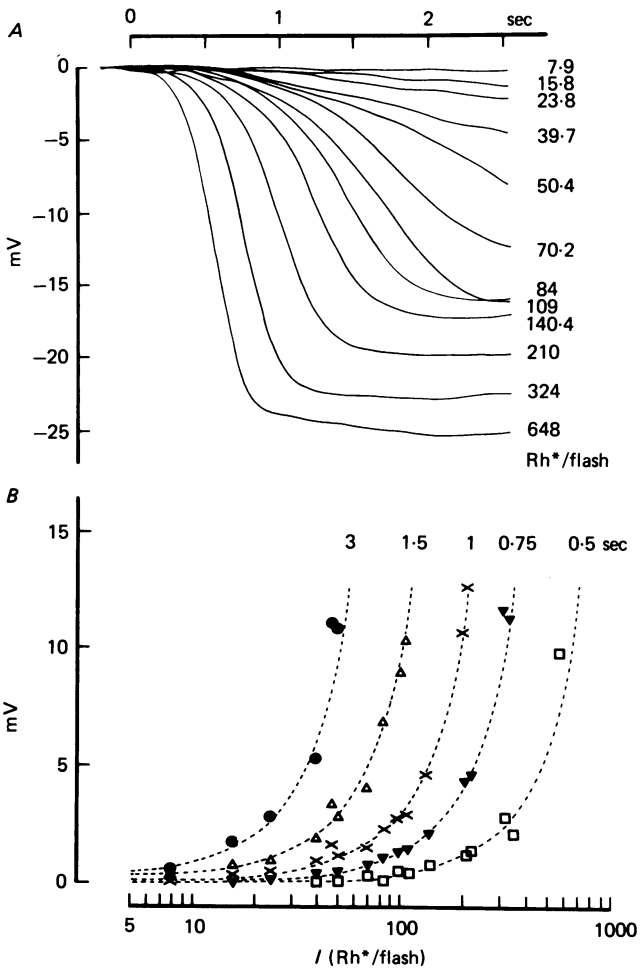

Fig. 5. Supralinearity in the presence of 50 μM-IBMX. *A*, voltage response to flashes the intensity of which is given by the figures near the trace. Each trace is the average of at least three responses. A given light intensity was obtained with appropriate neutral density filters and the flash duration was always shorter than 100 msec. Dark resting potential in control conditions was −50 mV and in the presence of 50 μM-IBMX was −33 mV. *B*, relation between amplitude of photoresponse and light intensity at fixed times. The dotted line is computed from the equation $I + 0{\cdot}1\, I^3$. The light intensity I is in dimensionless unit.

where I is the light intensity in arbitrary units. In general the relation between light intensity and amplitude of response at fixed times could be fitted with the equation:

$$\Delta V = C_1 I + C_2 I^2 + \ldots C_n I^n, \tag{3}$$

where $C_1 \ldots C_n$ are constants. In the case of 50 μM-IBMX the value for n was between

3 and 5. When data similar to those shown in Fig. 5*B* are plotted on log–log co-ordinates, it is evident that when ΔV is less than or about 1 mV, the relation between ΔV and I approaches linearity.

Recent studies on the electrical properties of isolated rods performed under voltage-clamp conditions (Bader, MacLeish & Schwartz, 1979; Attwell & Wilson, 1980) showed the existence of voltage- and time-dependent conductances which also affect the time course of response to dim light flashes (Detwiler, Hodgkin, McNaughton, 1980; Capovilla *et al.* 1980; Torre & Owen, 1981). These conductances, however, cannot account for the slow kinetics observed in the case of supralinearity. Regenerative photoresponses may be observed in rods after treatment with tetraethylammonium (TEA) (Owen & Torre, 1983 *b*). These responses are strongly dependent on the potential and the relation between ΔV and I changes in time. They are probably associated with a voltage- and time-dependent Ca^{2+} conductance with a negative resistance occurring between -27 and -35 mV. Very similar supralinear voltage photoresponses were observed when Ca^{2+} was reduced below 100 μM (Owen & Torre, 1983 *a*). By measuring the photocurrent with a suction electrode Yau, McNaughton & Hodgkin (1981) obtained similar results.

Effects of steady illumination on the response of rods treated with small amounts of IBMX

It is known that steady illumination can accelerate the kinetics of photoresponse in visual cells of a variety of animals (Fuortes & Hodgkin, 1964; Baylor & Hodgkin, 1974; Baylor *et al.* 1979). As mentioned above, small doses of IBMX have opposite effects on the kinetics of photoresponse to those of an illuminated background. It is interesting to see whether backgrounds of light of appropriate intensity antagonize the effects of IBMX and bring the kinetics back to control values. Fig. 6 shows two series of responses to flashes of different intensities (4 Rh*/rod in *A* and *B* and 8 Rh*/rod in *B*) in normal Ringer solution with no background illumination (dashed lines) and in the presence of 5 μM-IBMX in both dark-adapted conditions and with different background illuminations (continuous lines). The intensity of background illumination expressed as Rh*/rod . sec is given by the numbers on the right. It can be seen that both the increased sensitivity and the slowed kinetics could be reversed by steady background illumination when still in the presence of IBMX. However, the intensity of steady light necessary to restore kinetics back to control values was on average twice that needed to restore sensitivity back to normal values. Superimposed records at the bottom show that when the responses are scaled to the same peak amplitude it is always possible to find a background intensity that accelerates the kinetics of the response in IBMX back to control conditions.

Effects of small amounts of IBMX on flash sensitivity

In normal conditions, the flash sensitivity of a toad rod is approximately halved by a light background equivalent to about 5 Rh*/rod (Fain, 1976). The results of similar measurements performed in both control Ringer solution and in the presence of 5 μM-IBMX are illustrated in Fig. 7. In *A* the desensitization $1-{}_{\mathrm{l}}S_{\mathrm{f}}/{}_{\mathrm{d}}S_{\mathrm{f}}$ (${}_{\mathrm{d}}S_{\mathrm{f}}$ is the flash sensitivity in dark-adapted conditions; while ${}_{\mathrm{l}}S_{\mathrm{f}}$ the sensitivity in the presence of background illumination) is plotted as a function of the intensity of the conditioning background in both control conditions (open symbols) and in the presence of

5 μM-IBMX (filled symbols). It is seen that the desensitization induced by dim backgrounds is significantly more pronounced in the presence of IBMX than in control conditions, i.e. sensitivity is halved by steady lights equivalent to about 2 Rh*/sec.

With increased concentrations of IBMX (10–20 μM) the intensity of the light background that induced half desensitization decreased. At concentrations between 5 and 20 μM-IBMX, the efficacy of a background in reducing the flash sensitivity was

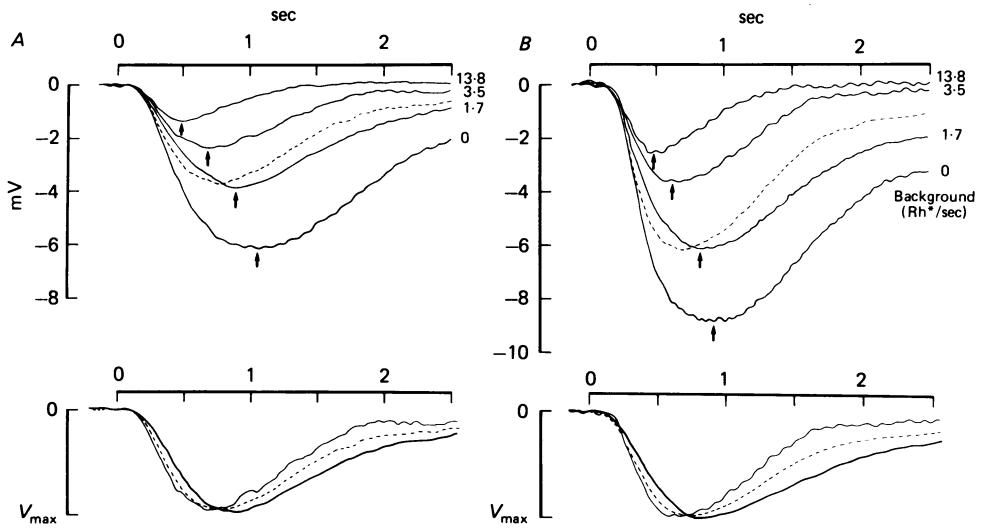

Fig. 6. Voltage responses to a flash of light equivalent to 4 Rh* (*A*) and to 8 Rh* (*B*) in the presence of 5 μM-IBMX and with different intensities of steady background illumination. Upper panel: dashed line is the voltage response observed in dark-adapted conditions in the control solution. Continuous lines are voltage responses to the same flash of light in the presence of 5 μM-IBMX, superimposed on steady backgrounds of light, the intensities of which (in Rh*/sec) are indicated by the numbers to the right of each record. Each trace is the average of at least eight responses. Lower panel: control response (dashed line) and responses in 5 μM-IBMX superimposed on backgrounds of light equivalent to 1·7 Rh*/sec (thick trace) and to 3·5 Rh*/sec (thin trace) scaled to the same peak response. Dark resting potential in control Ringer solution and in 5 μM-IBMX was −47 and −45 mV, respectively.

proportional to the increase in the time-to-peak of responses evoked by dim flashes. Baylor *et al.* (1980) have recently shown that the relation between flash sensitivity S_f and time-to-peak approximately satisfies the equation:

$$S_f = At_{pk}^n, \tag{4}$$

where n is between 2 and 3. Eqn. (4) was originally obtained by Fuortes & Hodgkin (1964) for a chain of low-pass filters with $n+1$ amplifying stages. The value of the constant A depends on the gain of the amplifying stages. The relation between sensitivity and time-to-peak is reported in Fig. 7*B* for rods in both control conditions and rods treated with 5 μM-IBMX. The experimental points obtained in control conditions and those in the presence of IBMX do not fit the same curve. The experimental points obtained in the presence of IBMX appear consistently shifted

to the right, as if the value of the constant A was changed. In contrast, the value of n does not seem changed, as is apparent when data from the same cell are compared. This observation suggests that in the presence of IBMX the number of slow stages is not modified. As mentioned earlier, the intensity of steady light necessary to restore sensitivity to its original levels is not sufficient in order to restore the kinetics to the

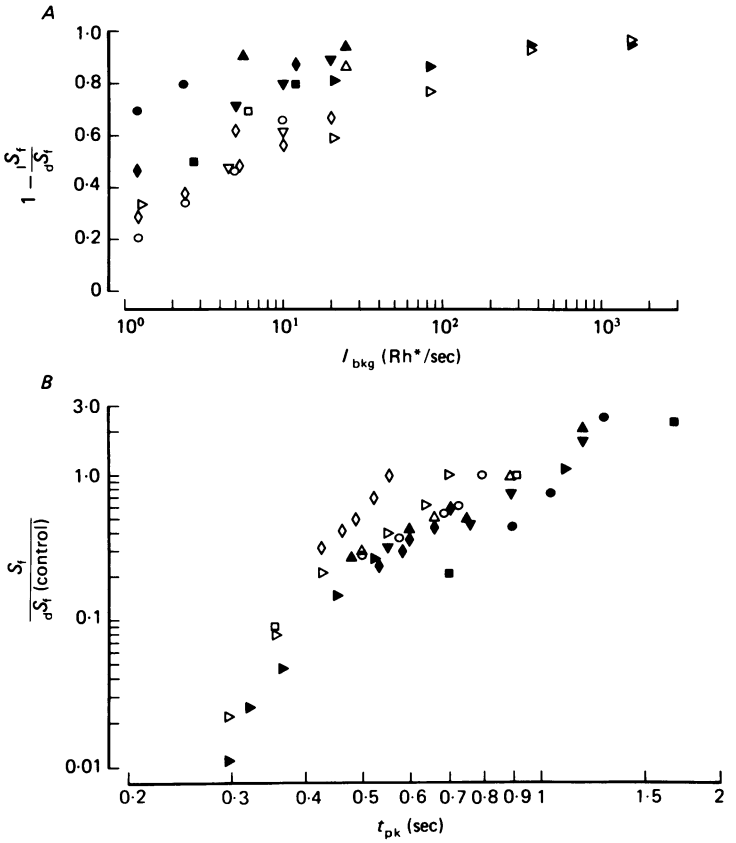

Fig. 7. A, dependence of desensitization $(1-{}_1S_f/{}_dS_f)$ on a steady background I_{bkg} in control condition (open symbols) and in the presence of 5 μM-IBMX (filled symbols). Collected data from six cells. B, relation between sensitivity and time-to-peak (t_{pk}) in control condition (open symbols) and in the presence of 5 μM-IBMX (filled symbols). Sensitivity in control condition has been normalized to 1. Collected data from six cells. In A and B same symbol indicates same rod. ${}_1S_f$: sensitivity in the presence of background illumination; ${}_dS_f$: sensitivity in dark-adapted conditions.

control values. This is equivalent to saying that the value of the constant A is reduced in the presence of IBMX (see eqn. (4)).

Effects of steady light in the presence of high concentrations of IBMX

An interesting consequence of operating in the supralinear range is that a dim background of light may enhance responses to dim flashes. Fig. 8 illustrates responses to a dim test flash (2 Rh*) from a rod bathed in 50 μM-IBMX. A illustrates responses

to the test flash delivered in darkness; *B* illustrates responses to an identical flash superimposed on a background of light of increasing intensity. Under these conditions the light background enhanced rod sensitivity instead of producing the usual desensitization. It should be emphasized that the dimmest background, corresponding to about 0·3 Rh*/sec, produced negligible shifts in the membrane potential thus

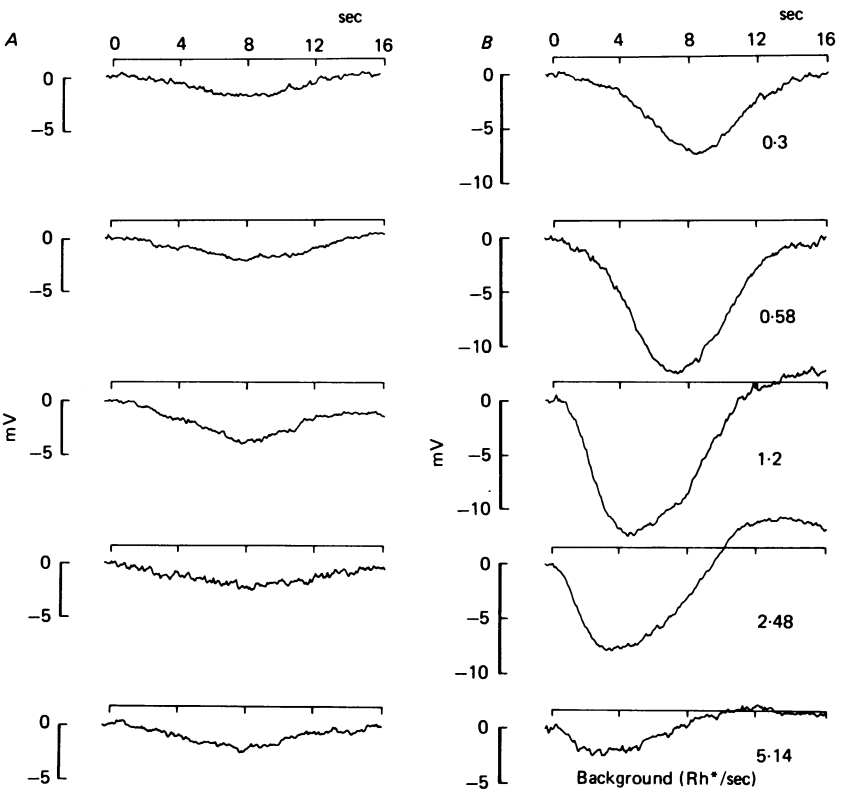

Fig. 8. Light sensitization in the presence of 50 μM-IBMX. On the left control responses in dark-adapted conditions to a flash of light equivalent to 1·2 Rh*. On the right responses to a flash of light equivalent to 1·2 Rh* superimposed on steady backgrounds of light, the intensity of which is indicated by the figures near the trace. Each trace is the average of at least three responses. The dark membrane potential in Ringer solution was −50 mV and −35 mV in the presence of 50 μM-IBMX. The steady backgrounds of light hyperpolarized the membrane potential by 0·5, 1, 3, 8 and 15 mV, starting from the weakest steady light.

indicating that voltage-dependent conductances do not account for this phenomenon. In the example shown in Fig. 8, the intensity of the background illumination that induced the largest increase in flash response was 1·2 Rh*/sec. Backgrounds brighter than 1·2 Rh*/sec were progressively less effective in enhancing the flash response and at intensities above 6 Rh*/sec they induced the usual desensitization.

Backgrounds of appropriate intensity may reverse the supralinear behaviour back to normal. Fig. 9 illustrates responses to flashes of increasing intensity from a rod treated with 50 μM-IBMX. It can be seen that supralinearity evident in flash responses obtained in the absence of background illumination (*A*), is suppressed by

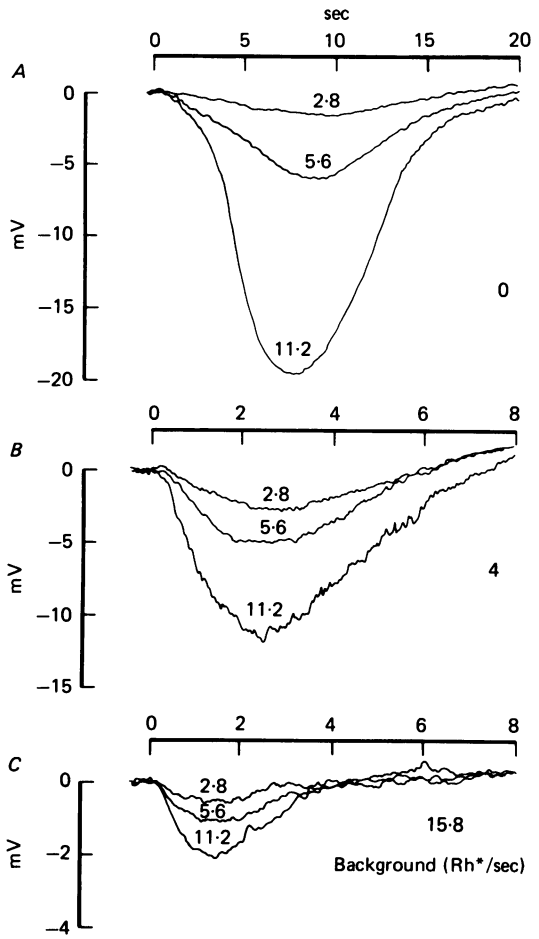

Fig. 9. Effect of steady light on supralinear behaviour in the presence of 50 μM-IBMX. Voltage response to flashes of light eqivalent to 2·8, 5·6 and 11·2 Rh*, respectively, in the presence of 50 μM-IBMX. Superimposed on steady lights equivalent to 0 (*A*), 5 Rh*/sec (*B*) and 15·8 Rh*/sec (*C*). Each trace is the average of at least three responses. Dark membrane potential in control conditions was −51 mV and in the presence of 50 μM-IBMX was −34 mV; the two light backgrounds induced a steady hyperpolarization of 7 and 17 mV, respectively.

exposing the rod to a background of 4 Rh*/sec (*B*) and completely abolished by a background of 15·8 Rh*/sec (*C*).

Antagonistic effects of light and IBMX on the time-to-peak of light responses

The antagonistic effects of light backgrounds and IBMX on the time-to-peak of photoresponse to dim flashes of light is summarized in Fig. 10. Here, the time-to-peak is plotted as a function of the intensity of steady illumination in control conditions (*A*) and in the presence of 5 (*B*), 10 (*C*) and 50 (*D*) μM-IBMX. In the presence of 5 μM-IBMX the control value of time-to-peak could be brought back to normal by exposing the rod to a background of about 8 Rh*/sec; while, in the presence of

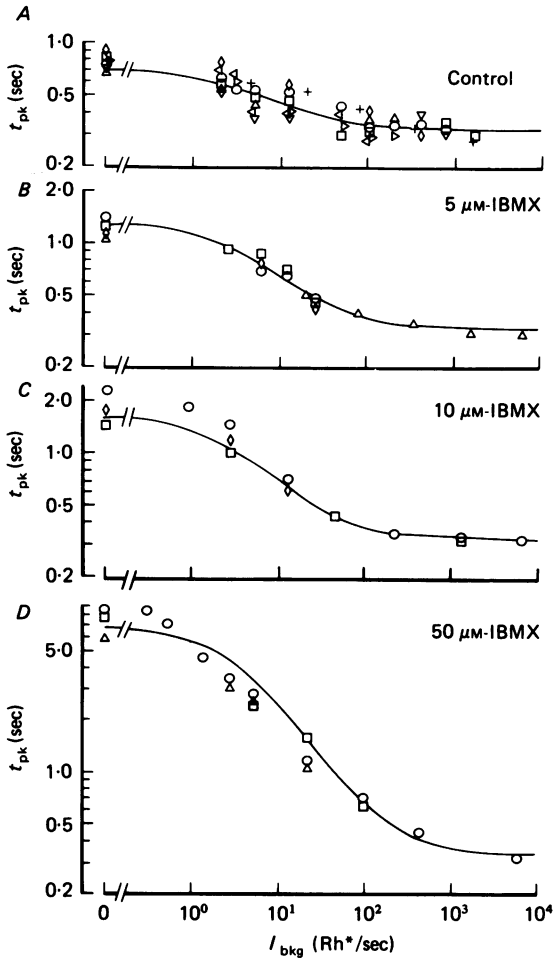

Fig. 10. Relation between time-to-peak (t_{pk}) of dim flash responses and intensity of a steady background I_{bkg} in control condition (*A*), in the presence of 5 μM-IBMX (*B*), in the presence of 10 μM-IBMX (*C*) and in the presence of 50 μM-IBMX (*D*). In *A*, *B* and *C* the time-to-peak was measured in the linear range whereas in (*D*), because of supralinearity, the criterion used was to measure the time-to-peak of responses smaller than 3 mV when the intensity of steady background was below 15 Rh*/sec. At brighter backgrounds the supralinearity was suppressed and the linear range criterion was adopted again. The continuous lines have been drawn from eqn. (5) with parameter values (for explanation see Text): $t_{min} = 330$ msec; $A_3 = 0{\cdot}2$ sec/(Rh*); $A_2 = 3$ μM; $A_1 = 1{\cdot}1$

50 μM-IBMX, the background intensity necessary to revert the time-to-peak back to control values is about 80 Rh*/sec. A minimum (t_{min}) value of the time-to-peak (about 350 msec) is obtained in the presence of bright backgrounds irrespective of the level of IBMX present in the perfusate.

The experimental points of Fig. 10 are fitted by the equation:

$$t_{pk} = t_{min}\frac{B + A_1(1 + [\text{IBMX}]/A_2)}{B};$$
$$B = 1 + A_3\, I_{bkg}, \qquad (5)$$

where A_1, A_2 and A_3 are constants, and I_{bkg} the intensity of the steady background (in Rh*/sec). The continuous lines drawn in Fig. 10 were obtained by means of eqn. (5) with the parameter values reported in the caption. The eqn. (5) is suggestive of an enzymatic reaction competitively inhibited by IBMX and in which the concentration of one of the cofactors increases with I_{bkg}. Among the possible physical models that may explain eqn. (5), it seems interesting to consider one in which: (1) the rate of reaction α_t of scheme (1) is proportional to the velocity of an enzymatic reaction with maximal velocity V_{max} and half activation K_m; (2) the enzyme of this reaction can be competitively blocked by IBMX with a dissociation constant equal to K_i; (3) the concentration of one of the cofactors (C) of this reaction increases with light according to $C = C_d + HI_{bkg}$, where C_d is the cofactor concentration in darkness and H is a constant. From these three hypotheses we obtain:

$$t_{pk} = K\frac{CS + K_m(1 + [\text{IBMX}]/K_i)}{CS}, \tag{6}$$

where K is a constant and S is the substrate concentration of the enzymatic reaction. Eqn. (6) bears the same dependence on IBMX and I_{bkg} as eqn. (5).

TABLE 1. Drug concentration that induces a two-fold increase in the time-to-peak of rod photoresponses in the linear range

Substance	Concentration (μM)
IBMX	3±1
Papaverine	7±2
RO 20–1724	20±5
Theophylline	120±20
Caffeine	300±30

Effects of different phosphodiesterase inhibitors

Expansion of the photoresponse time scale and supralinearity were observed using a variety of compounds known to act as phosphodiesterase inhibitors. We analysed the effects of both xanthine (caffeine, theophylline) and non-xanthine compounds (papaverine, RO 20–1724, diazepam, chlorpromazine and trifluoperazine). The drug concentration that induced a 50% increase in the t_{pk} of responses obtained in the linear range is reported in Table 1. IBMX is the most effective compound followed by papaverine, RO 20–1724, theophylline and caffeine with relative effectivities of 1, 1/2, 1/7, 1/40 and 1/100, respectively. Chlorpromazine, trifluoperazine and diazepam did not appreciably affect the properties of rod photoresponse even when applied at concentrations of 0·1–0·5 mM for 15 min. This observation is consistent with the data reported by Brodie & Bownds (1976) showing that osmotic effects occurred in rod outer segments exposed to papaverine, IBMX and RO 20–1724, but not in outer segments exposed to chlorpromazine. It may possibly be argued that the drug poorly penetrates the cell membrane or alternatively that the rod phosphodiesterase is calmodulin independent.

DISCUSSION

The effects of phosphodiesterase inhibitors on both kinetics and sensitivity of rod photoresponse described in this paper are probably the result of the inhibition of the hydrolytic activity of phosphodiesterase, since it is unlikely that all these drugs have similar side effects.

There is evidence to show that the time course of the current response to light may also depend upon the Na^+–Ca^{2+} exchange (Yau *et al.* 1981). The effect of 50 μM-IBMX on the kinetics of photoresponse, however, is 5–15 times larger than that induced by a drastic reduction of the Na^+ gradient (Yau *et al.* 1981; Torre, 1982).

If, in order to explain the time course of response to dim flashes, we use scheme (1), then an expansion of the time scale of the photoresponse is equivalent to a reduction of the reaction rates α_i all by the same factor. Since IBMX induces an expansion of the time scale of the dim flash response, one may consider the possibility that the slow reactions indicated in scheme (1) all depend, to some extent, on an enzymatic activity inhibited by IBMX.

As shown in the Results, the interaction between IBMX and time course of photoresponse may be quantitatively accounted for by an empirical relation (eqn. 5) which, together with the available knowledge on rod outer segment chemistry, suggests that the chemical reactions of scheme (1) could be catalysed by an enzyme inhibited by IBMX and the activity of which is enhanced by a light-activated cofactor. Since light leads to the activation of cyclic GMP phosphodiesterase in rods and the hydrolysis of cyclic GMP seems to be involved in phototransduction, it is tempting to identify cyclic GMP hydrolysis by phosphodiesterase as the reaction indicated in eqn. (6). Such an interpretation, however, implies that a steady background of light activates phosphodiesterase by affecting the K_m of the reaction rather than increasing the V_{max} as suggested by other evidence (Miki *et al.* 1973; Sitaramaya, Virmaux & Mandel, 1977).

A well known consequence of light adaptation is the shortening of the time scale of light response. This has been explained as due to an acceleration in the rate constants of the sequence of transformations of scheme (1). Since inhibition of phosphodiesterase mimics a 'negative light', the acceleration of cyclic GMP hydrolysis, induced by light, could be identified as the physical event underlying the changes in kinetics of photoresponse observed during light adaptation.

Supralinearity

As already mentioned, when rods are bathed in a solution containing small amounts of phosphodiesterase inhibitors, it is possible to observe supralinear behaviour and light sensitization. These two phenomena are likely to originate from the same mechanism, that we will term 'supralinearity'.

Supralinearity may be simply explained as due to a large increase of the total light sensitive conductance $\bar{G}_l$ assuming that the relation between the fraction of blocked channels and light intensity I, is steeper than the Michaelis–Menten relation (Lamb, McNaughton & Yau, 1981). Owen & Torre (1983 *a*) have shown that if the ratio

between the fraction of blocked light-sensitive channels and light intensity is

$$\frac{\bar{G}_l - G_l}{\bar{G}_l} = 1 - e^{-I}, \tag{7}$$

where G_l is the light-sensitive conductance in light, the normalized voltage response depends on I,

$$\frac{\Delta V}{\Delta V_{max}} = \frac{1 - e^{-I}}{1 + Ke^{-I}}, \tag{8}$$

where $K = \bar{G}_l/G$ which is the ratio between the total light-sensitive conductance $\bar{G}_l$ and the shunting conductance G. By plotting eqn. (8) for various values of K, it is clear that supralinearity occurs when K is larger than 3. Owen & Torre (1983 *a*) were able to explain the supralinearity observed in low extracellular Ca^{2+} (about 30 μM) with a value of K equal to 10. In order to explain the supralinearity observed in the presence of 50 μM-IBMX, however, the value of K should be increased from about 1 (in control Ringer solution) to a value larger than 50. Such a dramatic increase of the membrane conductance is not supported by input impedance measurements. The supralinearity explained by eqn. (8) requires no co-operativity between the effects of absorbed photons and can be termed as 'electrical supralinearity'. A simple way to consider co-operativity between absorbed photons is to use the 'multiple hits theory, well known in radiation biology (Zimmer, 1961). Let us assume that light produces a series of events that are Poisson distributed with an average λ proportional to the light intensity $\lambda = \gamma I$. Then, if the occurrence of at least a single event leads to the closure of a light sensitive channel, we obtain eqn. (7). But, if the occurrence of at least n events is necessary to close a channel, we obtain:

$$\frac{\bar{G}_l - G_l}{\bar{G}_l} = 1 - e^{-\gamma I}\left(1 + \gamma I + \frac{(\gamma I)^2}{2!} + \ldots \frac{(\gamma I)^{n-1}}{(n-1)!}\right). \tag{9}$$

Eqn. (9) provides a good mathematical model to explain supralinearity without a high value of K. This kind of supralinearity can be termed as 'chemical supralinearity'. Although this cannot be taken as a conclusive argument, the present results suggest the existence of co-operativity between photons in the process of phototransduction when phosphodiesterase is partially inhibited.

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