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Photodynamic effects of erythrosine on the smooth muscle cells of guinea-pig taenia coli

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1 Photon activation of the halogenated fluorescein derivative erythrosine caused a marked calcium-dependent contraction of the smooth muscle cells of the guinea-pig taenia coli superfused *in vitro*. Neither high intensity illumination alone (up to 5×10^4 lux) nor erythrosine alone (up to 2×10^{-4} M) altered the tone of the taenia or its ability to respond to carbachol (5×10^{-5} M); photo-irradiation of erythrosine before tissue contact was also ineffective.

2 The magnitude of the photodynamic contraction was dependent upon the concentration of erythrosine, the intensity and wavelength of the incident light, and the presence of oxygen; indirect effects via neurotransmitter release or cyclo-oxygenase activation were specifically excluded.

3 The photodynamic response was blocked by zero-$[Ca]_o$ and addition of EGTA (1 mM) but not by omission of $[Mg]_o$ or a decrease in $[Cl]_o$ or $[Na]_o$. D600 (methoxyverapamil) 10^{-5} M, or a ten fold increase in $[Mg]_o$, to 11.3 mM, partly inhibited the photodynamic contraction at low, but not high, light intensities.

4 These observations are consistent with the following sequence of events: (i) photo-activation of the erythrosine molecule, (ii) the generation of highly reactive singlet oxygen, (iii) local peroxidation of cell membrane proteolipid, (iv) increased membrane permeability to Ca^{2+}, (v) the influx of Ca^{2+} and, (vi) muscle contraction.

5 It is concluded that the photodynamic action of erythrosine presents a novel method for modulation of membrane calcium permeability, and hence $[Ca]_i$, not only in smooth muscle but possibly in other cells as well, e.g., secretory, epithelial and myocardial cells.

Introduction

The halogenated fluorescein derivative Erythrosine B (erythrosine) has been found to produce a wide variety of effects *in vitro*: it inhibits neurotransmitter uptake in rat brain slices (Logan & Swanson, 1979) and alters dopamine transport in synaptosomes (Lafferman & Silbergeld, 1979; Mailman *et al.*, 1980), inhibits rat brain Na K-ATPase (Silbergeld, 1980) and $^{86}Rb^+$ uptake in the guinea-pig heart (Bihler *et al.*, 1981), modifies neurotransmitter release from the frog neuromuscular junction (Augustine & Levitan, 1980; 1983a,b), and mediates oxidation-reduction events occurring in brain membranes (Floyd, 1980). No common mechanism of action has been established for these diverse effects although from observations on artificial membranes it has been claimed that erythrosine acts as a simple ionophore capable of mediating transmembrane movements of ionized calcium (Columbini & Wu, 1981).

The present experiments were designed to assess the actions of erythrosine on the guinea-pig isolated taenia (caecum) coli, a smooth muscle preparation in which the influx of Ca^{2+}, associated with well-defined Ca^{2+}-dependent action potentials, is closely coupled to contraction. In this study the erythrosine-dependent generation of highly reactive singlet oxygen not only fully accounts for the functional responses of the taenia coli we have observed, but at the same time it provides a novel photochemical approach for exploring the molecular organization and operation of membrane ionic permeability mechanisms and calcium transport in smooth muscle and other cells.

A preliminary communication of some of these findings was presented to the British Pharmacological Society in Cambridge, April, 1983 (Braithwaite *et al.*, 1983).

Methods

Superfusion of taenia coli

Guinea-pigs (200–400 g) of either sex were killed by cranial percussion and exsanguination. Strips of taenia were obtained by dissection from the caecum and superfused in a system similar to that described by Brading & Sneddon (1980) and Lowe *et al.* (1981). Pieces of taenia approximately 1 cm in length were secured at one end of the base of glass superfusion chambers (vol, 1 ml) while the other ends were fastened to transducers (tension, 1 g) located directly over the chambers for the isotonic recording of contraction. The superfusate was pumped by a Technicon multichannel pump from glass reservoirs via polythene tubing (i.d. 1.5 mm) placed in a water bath (Grant SB-1) at 35°C before entering the base of the chambers; the effluent superfusate was discarded. Flow rates were set to approximately 1 ml min^{-1}. Initially two, and later four, superfusion systems were run in parallel; contractions were recorded with Bryans Southern Instruments 28000 chart recorders.

Solutions

In the majority of experiments a modified Krebs-Henseleit solution was used of the following ionic composition (mmol l^{-1}): NaCl 118, $CaCl_2$ 2.56, KCl 4.7, $MgCl_2$ 1.13, NaH_2PO_4 1.15, $NaHCO_3$ 25, supplemented with 5.6 mM D-glucose. The solution was bubbled with 95% O_2 and 5% CO_2 to give a final pH of 7.4. In some experiments 95%N_2 and 5%CO_2 was used as the gas phase and the pH maintained at 7.4. Omissions from or additions to, the Krebs solution are indicated where appropriate. Isosmotic substitutions of NaCl by choline chloride or sodium propionate were made in some experiments; in others isosmotic Tris or sucrose was substituted completely for $[Na]_o$ in the solution, the gas phase being 100% O_2.

Light intensity

All experiments were performed in a partially darkened room. Controlled illumination of the tissue was provided by a Schott KL 150 quartz-halogen light source equipped with a heat filter (KG1) and producing a maximum intensity of 14×10^4 lux at 640 nm. A fibre optic probe provided a 60° illumination field of uniform intensity such that up to four superfusion baths could be exposed simultaneously to the same light source. Absolute light intensities were determined to high accuracy (±3%) with a Hagner Model E-2 Digital Luxmeter. No heating of the superfusion baths by illumination was measurable even at maximal light intensities.

For determination of the wavelength 'action spectrum' a series of sharp-cut filters (G.K. Turner Associates, Palo Alto, Calif., U.S.A.) of defined transmission characteristics was interposed between the optic probe and the preparation. The wavelength range from 520 to 565 nm was scanned sequentially by the bandwidth limits set by six different filters, namely 2A-12, 2A-15, 16, 22, 65A and 58. The absorption spectrum for aqueous erythrosine was determined by a Pye-Unicam SP8 – 200 UV/Vis spectrophotometer.

Experimental procedures

In all experiments tissue preparations were allowed to equilibrate for 30 to 45 min. Reproducible responses to carbachol, 5×10^{-5} M (added to the superfusion fluid for 10 s) could usually be obtained (variation $< \pm 3\%$) during this time: any preparation not capable of a consistent response was replaced. Similar contractions were elicited by isotonic KCl (for 10 s). The magnitude of the photodynamic contraction in the presence of erythrosine is expressed as a percentage of the contraction elicited by carbachol, 5×10^{-5} M alone (designated C_1 in Figure 1).

Statistical analysis

Results are expressed as the mean ± s.e.mean. Statistical significance was determined by application of Student's *t* test. Rates of inhibition of the carbachol and KCl responses and the correlation between photodynamic contraction magnitude and latency to onset of contraction were determined by least squares analysis.

Materials

All standard chemicals were of 'Analar' or comparable purity. Other chemical agents were obtained as follows: atropine sulphate, BDH Chemicals Ltd, Poole Dorset; carbachol, Koch-Light Laboratories Ltd, Bucks; cinanserin, Sandoz Ltd, Basle; D600 (methoxyverapamil), Knoll A.G., Ludwigshaven; Erythrosine B (sodium salt), indomethacin, Rose Bengal (sodium salt) and Eosin Y, Sigma, Dorset; mepyramine maleate, May & Baker, Dagenham.

Results

Erythrosine-sensitized photodynamic contraction

Treatment of the smooth muscle of the guinea-pig taenia coli with erythrosine in a superfusion system *in vitro* produced, but only in the presence of illumination, an increase in resting tone. Neither high intensity illumination alone (up to 5×10^4 lux), nor ery-

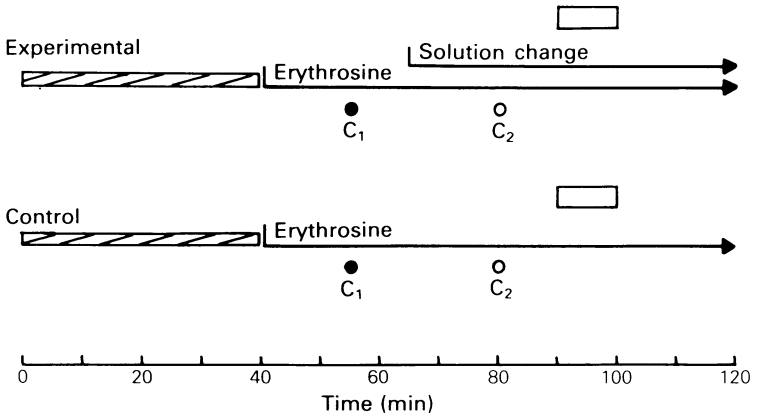

Figure 1 Erythrosine-sensitized photodynamic contraction experiments; schematic diagram of experimental procedure. After the stabilization period (shaded rectangles), erythrosine , 2×10^{-5} M, was added to the solution superfusing two or more preparations in parallel. Carbachol, 5×10^{-5} M, was applied (for 10 s) as indicated at C_1 and C_2. Any solution change or drug addition was made in one preparation, the other serving as a control. Each preparation was illuminated for 10 min at a given intensity (open rectangles).

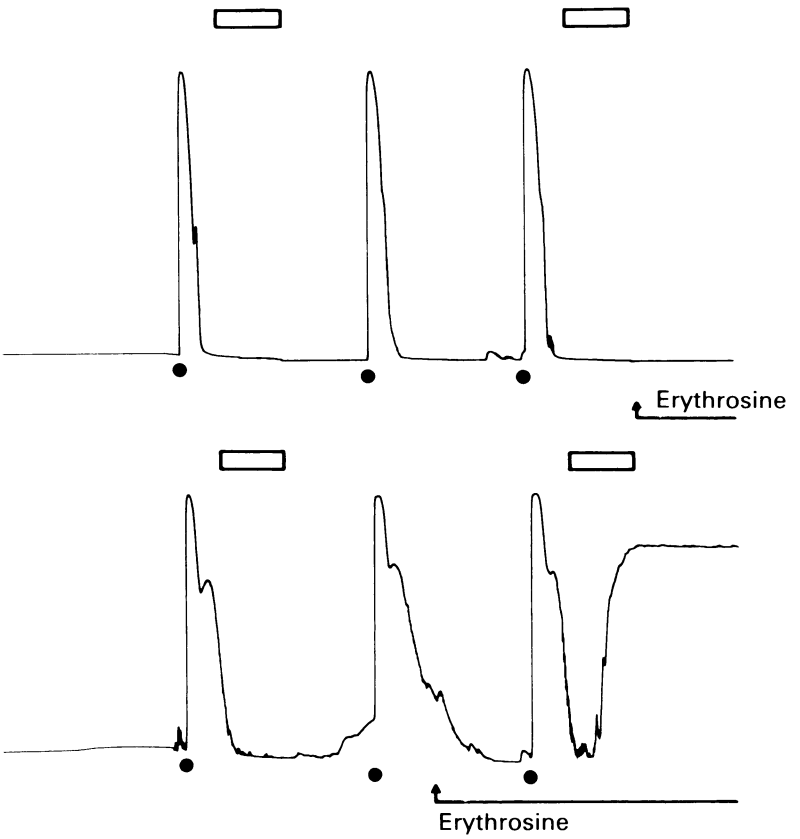

Figure 2 Effects of illumination and erythrosine, either alone or in conjunction, on the tone and evoked responses of taenia coli. Two preparations (upper and lower panels) superfused in parallel. Contractions to carbachol, 5×10^{-5} M, (●), and exposure to erythrosine, 2×10^{-5} M, at the arrows; illumination at 1.7×10^4 lux for 10 min (open rectangles).

throsine alone (up to 2×10^{-4} M) had any effect on the tone of the taenia preparations, or the ability of the muscle to respond to the test agonist, carbachol (Figure 2). Other control experiments demonstrated that photon irradiation of erythrosine solutions before tissue contact similarly elicited no muscle response. However, illumination of the smooth muscle during a period of erythrosine exposure produced a contraction with a magnitude dependent on light intensity (Figure 3) and dye concentration (Figure 4). The log (intensity)-response curve was linear over the range of 2×10^3 to 2×10^4 lux. The magnitude of the photodynamic contraction and the latency between the onset of illumination and initiation of contraction (Figure 5) were also closely correlated ($r = -0.91$). The results of Figure 4 demonstrate that the magnitude of the photodynamic contraction increased markedly between erythrosine concentrations of 1×10^{-6} and 2×10^{-5} M, some effect being evident at concentrations as low as 2×10^{-8} M. At higher concentrations, i.e. $> 5 \times 10^{-5}$ M the response may be decreased by self-quenching molecular interactions and the reduced formation of reactive intermediates (see below).

The requirement for both photon energy and the presence of dye-sensitizer together in order to elicit a photodynamic effect on smooth muscle indicates the involvement of an erythrosine-transient or free radical species of short half-life since pre-irradiation of erythrosine itself was ineffective. Halogenated fluorescein derivatives of the erythrosine type are known to exist in an excited triplet state and, by electron orbital transfer, give rise to highly reactive singlet oxygen (Foote, 1968).

If the local photo-activation of erythrosine underlies photodynamic contraction of the muscle and the two events are causally related then both should depend upon a similar and specific region of the incident radiation spectrum. By confining the radiation incident upon the superfused tissue ($n = 6$) successively to localized spectral regions with sharp-cut filters of known band-pass and transmission characteristics (see Methods), an 'action spectrum' was established between 527 and 562 nm with a spectral peak for photodynamic contraction in the region of 538 nm. This represents only a small bathochromic or 'red' shift in the absorbance maximum of 526 nm for erythrosine in water and corresponds exactly to the absorbance peak of 538 nm for erythrosine partitioned in a macromolecular matrix (Bourdon & Duranté, 1970).

A further important point is that removal of oxygen from the superfusing solution should inhibit the erythrosine-sensitized photodynamic contraction if singlet oxygen is involved. In fact, whilst removal of oxygen from Krebs solution by displacement with nitrogen had little effect on the contraction of the muscle to carbachol itself, it completely obliterated the photodynamic contraction normally elicited by erythrosine (Figure 6, Table 1). Re-introduction of oxygen 10 min after the illumination period gave no increase in tone, emphasizing that photon energy and oxygen are essential *together* with erythrosine if photodynamic interaction is to occur. Carbachol pro-

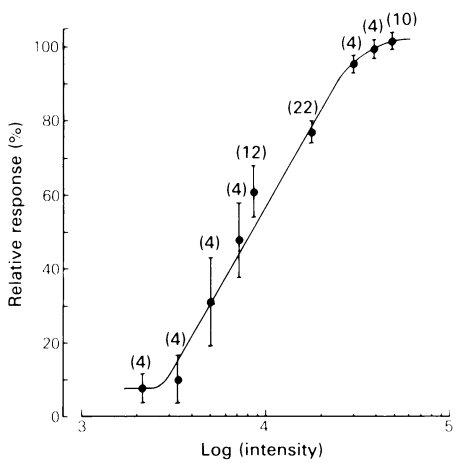

Figure 3 Relationship between the magnitude of the erythrosine-sensitized photodynamic contraction and the logarithm of the illumination intensity (lux). In each experiment the taenia was exposed to erythrosine, 2×10^{-5} M, for 50 min before illumination at a given intensity. Photodynamic contractions were measured relative to a reference response to carbachol, 5×10^{-5} M. Number of experiments in parentheses.

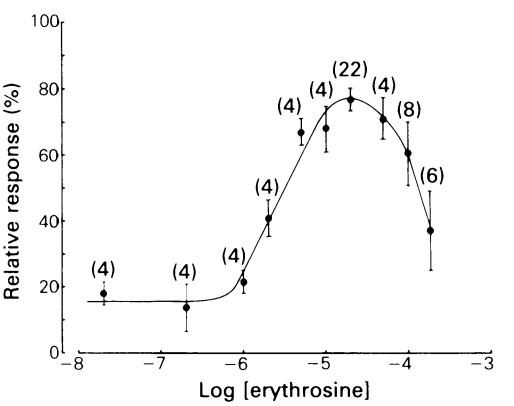

Figure 4 Effect of erythrosine concentration on the magnitude of the photodynamic contraction. Illumination was for 10 min, at 1.7×10^4 lux, and contractions were measured relative to a reference contraction to carbachol, 5×10^{-5} M. Number of experiments in parentheses.

Table 1 Effects of inhibitors on the erythrosine-sensitized photodynamic contraction of taenia coli

Inhibitor	*Carbachol response in presence of inhibitor†*	*Light intensity* ($\times 10^4$ lux)	*Photodynamic response† with erythrosine 2×10^{-5} M* *Inhibitor absent*	*Inhibitor present*	*Carbachol response after removal of inhibitor†*
Nitrogen	97.6 ± 0.9*	4.8	97.5 ± 0.4	0 ± 0***	99.8 ± 0.8
Dithionite (1 mM)	101.9 ± 1.9	1.7	86.8 ± 3.9	12.2 ± 4.2***	103.0 ± 1.6
zero-$[Ca]_o$	54.4 ± 8.5***	1.7	78.5 ± 6.1	0 ± 0***	—
		4.8	98.9 ± 4.3	24.6 ± 12.7***	—
zero-$[Ca]_o$ + EGTA (1 mM)	0 ± 0***	4.8	93.8 ± 0.9	0 ± 0***	—
D600 (10^{-5} M)	50.0 ± 8.3***	1.7	79.9 ± 7.8	43.4 ± 12.5*	—
		4.8	98.9 ± 9.8	96.7 ± 6.1	—
$[Mg]_o$ (11.3 mM)	99.4 ± 0.4	1.7	75.9 ± 10.5	3.5 ± 0.5***	—
		4.8	93.2 ± 2.9	71.8 ± 9.1	—

† Expressed as % of control carbachol response: mean ± s.e. ($n = 4$ to 10). Significantly different from control values; *$P < 0.05$, ***$P < 0.001$

duced no additional increase in tone in those (oxygenated) preparations in which the photodynamic contraction had reached a maximum, but it consistently produced a normal muscle contraction when N_2-exposure was followed by restoration of oxygen (Figure 6, Table 1). This demonstrates that the muscle still possesses full contractile capacity during and after N_2-exposure and indicates that it is a lack of oxygen which selectively prevents the photodynamic action of erythrosine rather than any non-specific effect of N_2 exposure.

Additional evidence confirming the generation of singlet oxygen as the predominant reactive intermediate in erythrosine-sensitized photodynamic ac-

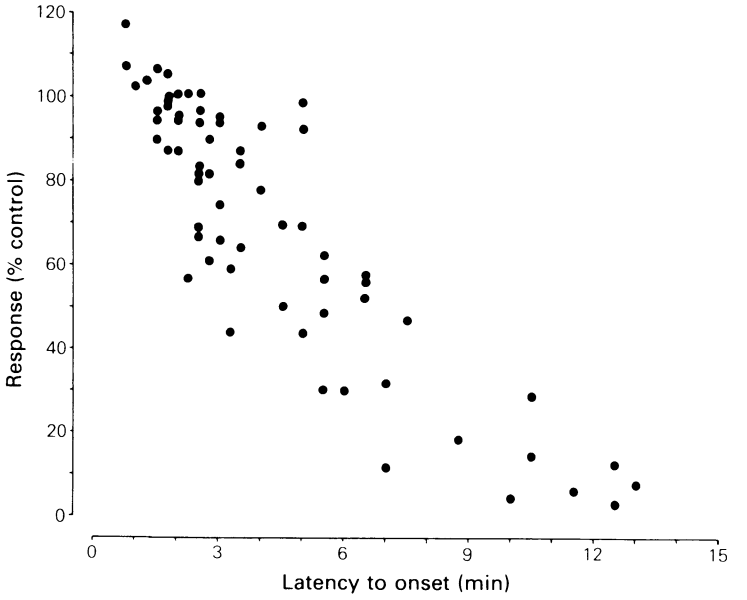

Figure 5 Relationship between the magnitude of the photodynamic contraction and the latency to onset of the contraction. Data from individual preparations of Figure 3. In these experiments the taenia was exposed to erythrosine, 2×10^{-5} M, for 50 min before illumination at a given intensity (see Figure 3). Photodynamic contractions were measured relative to a control response to carbachol, 5×10^{-5} M. Latency measured as the time which elapses from the start of a period of illumination until the muscle first begins to contract.

tion came from use of the potent antioxidant, dithionite (1 mM), which had no effect on the response of the muscle to carbachol but inhibited the erythrosine-induced response by 86% ($P<0.001$); see Table 1.

Effects of neurotransmitter antagonists on the erythrosine-sensitized photodynamic contraction

Antagonists were used to test for the possibility that the erythrosine-induced photodynamic contraction was elicited indirectly by neurotransmitter release or the mediation of arachidonate cyclo-oxygenase products. At concentrations which were found to inhibit markedly or block totally the action of the appropriate agonist, neither atropine (6×10^{-6} M), mepyramine (5×10^{-6} M); cinanserin (6×10^{-5} M), nor the prostaglandin synthesis inhibitor, indomethacin (10^{-4} M), had any effect on the erythrosine-sensitized photodynamic contraction ($n=3$ to 6 experiments for each antagonist). The local anaesthetic tetracaine (1 mM) was similarly without effect ($n=3$, $P>0.05$).

Effects of ionic substitutions on the erythrosine-sensitized photodynamic contraction

In order to analyse the ionic and cellular basis for the photodynamic response the concentrations of $[Ca]_o$, $[Mg]_o$, $[Na]_o$ and $[Cl]_o$ were modified.

The removal of magnesium from the extracellular fluid had no effect on either the carbachol- or erythrosine-induced contraction whereas omission of calcium decreased the carbachol contraction and markedly inhibited or blocked the photodynamic response (Figure 7a, Table 1). Addition of EGTA (1 mM) completely blocked both contractions (Figure 7b, Table 1). In each instance, restoration of calcium led to the appearance of a delayed contraction (Figure 7a) demonstrating the clear Ca^{2+}-

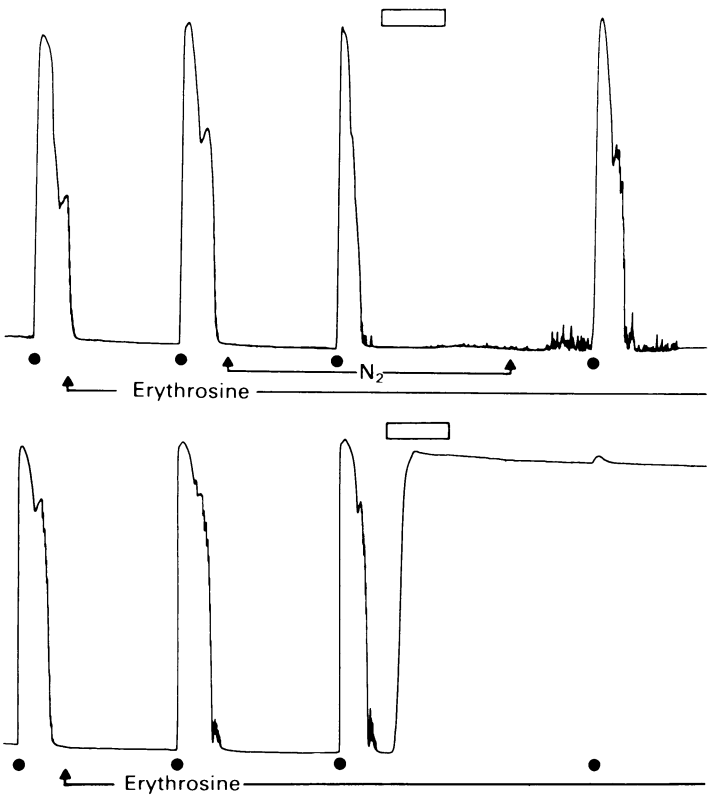

Figure 6 Effects of replacing oxygen in the solution with nitrogen (N_2) on the erythrosine-induced photodynamic contraction. Two preparations (upper and lower panels) superfused in parallel with erythrosine, 2×10^{-5} M, beginning at the arrows. Contractions to carbachol, 5×10^{-5} M (●) and illumination for 10 min at 1.7×10^{4} lux (open rectangles). Removal of oxygen and replacement with N_2 between the arrows in the upper record only. Note the normal response to carbachol after restoration of oxygen (upper panel).

dependence of the photodynamic response.

D600 (methoxyverapamil), a calcium antagonist which acts by direct competition with Ca^{2+} for entry at the voltage-dependent Ca^{2+}-channels in smooth muscle (Riemer *et al.*, 1974; Bolton, 1979) partly inhibited the photodynamic contraction at low but not high light intensities (Table 1). Similar but more pronounced effects were seen when an alternative means of blocking Ca^{2+} entry was used, namely increasing $[Mg]_o$ ten fold to 11.3 mM (Table 1).

In contrast to these effects, a decrease of $[Na]_o$ to 25 mM by choline substitution produced no inhibition of the photodynamic contraction, and although total removal of $[Na]_o$ by isosmotic substitution of Tris or sucrose did lead to some decrease in the photodynamic contraction ($n = 4$), the interpretation of these experiments is complicated by the fact that the carbachol contraction was also markedly inhibited. Total removal of $[Na]_o$ is known to have an action on the contractile process of taenia coli via indirect effects on calcium handling (Brading et al., 1980). In view of the Ca-dependence of the process it is highly likely that the inhibition of the photodynamic (and carbachol) contraction observed here is attributable to these secondary effects of sodium removal.

Finally, anionic substitution of $[Cl]_o$ by propionate, 118 mM, had no significant effect ($P > 0.05$) on either the response to carbachol or the photodynamic contraction ($n = 8$).

Erythrosine-sensitized photodynamic effects on contractions elicited by carbachol and KCl

As is evident from Figures 2, 6 and 7, carbachol-induced contractions were totally unaffected by light alone or erythrosine alone. However, in the presence of illumination and erythrosine together the contractions evoked by carbachol or by increasing $[K]_o$ were rapidly inhibited, the potassium response being at first briefly enhanced (Figure 8a). In a more systema-

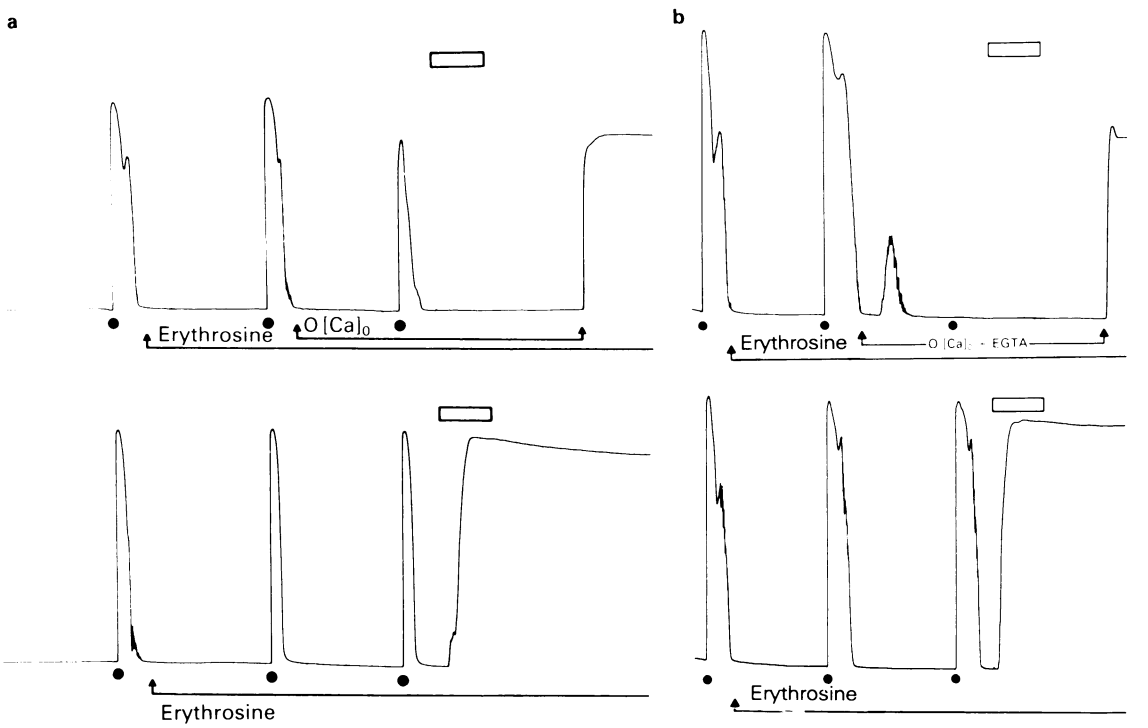

Figure 7 Effects of the removal of extracellular calcium (a) and the addition of EGTA, 1 mM (b) on the erythrosine-induced photodynamic contraction. Erythrosine, 2×10^{-5} M, present as indicated. Contractions to carbachol, 5×10^{-5} M (●) and illumination for 10 min at 1.7×10^4 lux (open rectangles). Note the restoration of the photodynamic contraction on reintroduction of normal $[Ca]_o$, upper panels. Each lower panel represents a control experiment run in parallel with that of the upper panel.

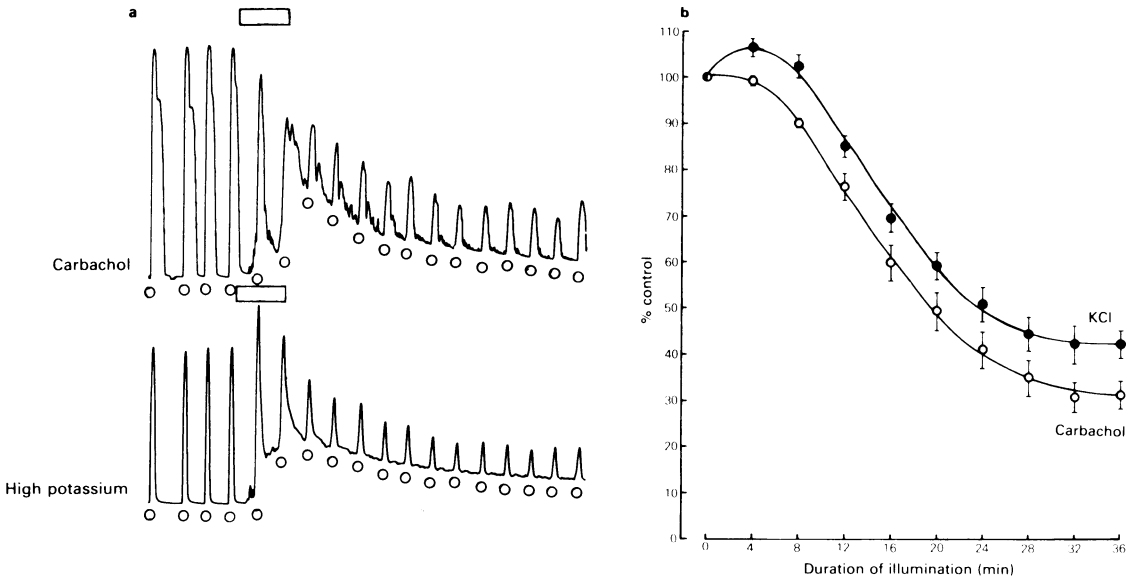

Figure 8 Erythrosine-induced photodynamic inhibition of responses to carbachol or increased $[K]_o$. (a) Responses elicited at 5 min intervals with carbachol, 5×10^{-5} M, (○) top panel or 10 × normal $[K]_o$ (i.e. 47 mM) (○) lower panel. Exposure to erythrosine, 2×10^{-5} M, throughout the experiment beginning 30 min before illumination for 10 min at 3×10^4 lux (open rectangles). (b) Exposure to erythrosine, 2×10^{-5} M for 18 min before beginning a series of alternate contractions to carbachol, 5×10^{-5} M, (○) and isotonic KCl (●). Illumination for 4 min at 10^4 lux preceded each carbachol and KCl response and was repeated until a total illumination time of 36 min had elapsed. The magnitude of the evoked contractions in the erythrosine-sensitized preparations ($n = 22$) is expressed as a percentage of the corresponding controls in the absence of illumination.

tic analysis of the action of erythrosine and illumination, the cumulative effects of repeated short illumination periods at low light intensity were tested for their inhibitory action on the carbachol and KCl evoked responses (Figure 8b). After the initial phase the rates of inhibition relative to the control responses were similar, for carbachol $2.6 \pm 0.2\%$ min^{-1} and for KCl $2.8 \pm 0.2\%$ min^{-1} ($n = 22$). In experiments similar in design to those of Figure 8b except that O_2 was removed and N_2 substituted in the gas phase, the contractions to both carbachol and KCl were significantly less inhibited ($P < 0.05$; $n = 8$) than in the presence of oxygen. This confirms that a similar molecular action underlies the erythrosine photodynamic contraction (Figure 6) and the erythrosine-sensitized photodynamic inhibition of the carbachol and KCl responses (Figures 8a,b).

Effects of other halogenated fluorescein derivatives

Comparison was made between erythrosine i.e. Erythrosine B (tetraiodofluorescein) and two other halogenated fluorescein derivatives, Rose Bengal (tetrachloro-tetraiodofluorescein) and Eosin Y (tetrabromofluorescein) for photodynamic action on taenia coli. Under similar conditions Rose Bengal was the most active compound, the potency ratio calculated from the 50% contractile response evoked by illumination being Rose Bengal (3.8) > erythrosine (1.0) > eosin Y (0.013).

Persistence of erythrosine's action

The experiments described so far refer to conditions in which the erythrosine (or other photochemical agent) was maintained present throughout the illumination period. In later experiments it was found that a photodynamic contraction could still be elicited 3 h or more after erythrosine had been removed from the superfusion fluid (Table 2). The tissue therefore retains the dye for prolonged periods in a state capable of photoactivation.

In this type of experiment, EGTA (1 mM) rapidly offset the photodynamic contraction when added after the contraction had reached its maximum.

Discussion

These results demonstrate that the magnitude of the erythrosine-induced photodynamic contraction of the taenia coli is dependent upon the concentration

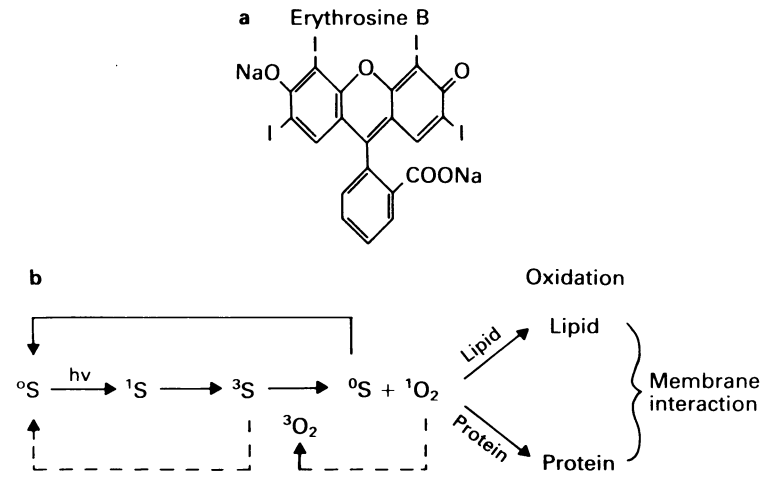

Figure 9 The structure of erythrosine (a) and the molecular mechanism for photodynamic action (b). See text for further details.

of erythrosine, the intensity and wavelength of the incident light, and the presence of oxygen. The proximity of the 'action' spectrum for photodynamic contraction to the absorption spectrum for aqueous erythrosine and far from the wavelengths of amino-acid and nucleotide absorption also clearly implicates photo-activation of the dye at an early stage in the photodynamic process. The photo-activation must be rapidly reversible and the molecular products of short half-life because pre-irradiation of erythrosine seconds before tissue contact was found to be ineffective, and the absorption spectra of the erythrosine solutions examined before and after eliciting a photodynamic contraction were identical. As already noted, a shift in the erythrosine 'action' spectrum peak from 526 to 538 nm coincides with that found for the binding of erythrosine to a protein macromolecular matrix (Bourdon & Durante, 1970). This suggests that bound erythrosine is particulary effective and, in view of the spatial and temporal limitations placed on the diffusion rates of an active species of short half-life, localization of erythrosine molecules at the cellular surface would certainly be more likely to initiate a photodynamic effect. With an octanol:water coefficient of 0.71 (Augustin & Levitan, 1980) erythrosine will partition into the low polarity environment of the cell membrane; this would explain why in the present experiments the smooth muscle cells of the taenia retained erythrosine even after a comparatively brief exposure to the dye. The bound erythrosine, which did not itself affect the carbachol response, could still be photoactivated several hours later (see Table 2).

Replacement of oxygen in the solution with nitrogen, a technique which successfully inhibits the photodynamic action of halogenated fluoresceins on

Table 2 Photodynamic response of taenia coli to light after erythrosine wash-out

Wash-out time before illumination	*Response to carbachol immediately before illumination*	*Photodynamic response after illumination†*
None	100	85.7 ± 2.7
30 min	99.6 ± 0.5†	92.6 ± 0.8
1 h	98.4 ± 0.3†	92.5 ± 1.5
2 h	99.3 ± 0.4†	83.9 ± 3.5
3 h	101.4 ± 1.6†	72.8 ± 9.8

All preparations were exposed to erythrosine 2×10^{-5} M for 25 min before wash-out commenced. Illumination for 10 min at 1.7×10^4 lux after the wash-out time indicated.

†Expressed as % of the control response to carbachol (5×10^{-5} M) obtained during the initial period of erythrosine exposure: mean ± s.e. ($n = 3$ to 6).

proteins in solution (Kepka & Grossweiner, 1973), totally and specifically inhibited the erythrosine-induced photodynamic contractions in the present experiments. Similarly sodium dithionite, a potent reducing agent which will decrease oxygen tension markedly, also inhibited the photodynamic action of erythrosine. All the evidence of our experiments is consistent with that for a number of different erythrosine-sensitized photodynamic systems *in vitro* all of which involve the generation of a highly reactive singlet oxygen molecule. In this process (Figure 9) the absorption of photon energy ($h\nu$) by the ground state erythrosine molecule (^0S) elevates an electron in the conjugated ring system of the molecule to the excited singlet state (^1S). The short-lived singlet state (^1S) decays rapidly ($10^{-11}-10^{-12}$ s) but a substantial number of molecules ($>50\%$) reach the excited triplet state (^3S) through an inter-system crossing process. The longer-lived ($>10^{-3}$ s) triplet state (^3S) interacts with ground state molecular oxygen ($^3O_2$) and through energy transfer creates excited singlet oxygen ($^1O_2$), (Grossweiner, 1969; Gollnick *et al.*, 1970). Although singlet oxygen has a comparatively short lifetime (10^{-5} s) it is sufficient to allow localized diffusion and interaction with membrane protein or lipid (Spikes & MacKnight, 1970; Chan, 1977; Pooler & Valenzeno, 1979).

These chemical events obviously occur with a rapid time constant and factors others than neurotransmitter release or prostaglandin generation, which we have specifically excluded, must underlie the latency of the photodynamic contraction. Light intensity and erythrosine concentration are variables which clearly will affect the latency of the response but the delay inherent in the excitation-contraction process itself may also be significant since the photodynamic contraction must involve either a direct activation of the contractile proteins or a time-dependent increase in $[Ca]_i$. In the normal process of contraction an increase in $[Ca]_i$ may be accounted for by release from an internal store (Casteels & Raeymaekers, 1979; Brading & Sneddon, 1980; Den Hertog, 1982), calcium influx due to calcium-dependent action potentials (Imai & Takeda, 1967; Brading *et al.*, 1969; Casteels & Droogmans, 1982) or some other modification of resting membrane permeability (Bolton, 1979).

The experiments with D600, a calcium antagonist effective in blocking spontaneous action potential generation and tonic contraction in the taenia (Bolton, 1979), showed a significant inhibition both of the carbachol-induced contraction and of the photodynamic contraction at moderate light intensities. At this same intensity, total removal of calcium from the solution, a procedure known to be effective in washing extracellular and non-sequestered intracellular calcium from the taenia (Brading & Sneddon, 1980), effectively abolished the photodynamic contraction. These observations rule out the possibility of a direct action on the contractile proteins and suggest an effect localized to calcium permeability at the cell membrane. Reintroduction, after the illumination period, of normal calcium levels into the calcium-deprived preparations initiated a rapid reappearance of the photodynamic contraction, suggesting that photodynamic action serves to alter membrane properties such that the presence of normal $[Ca]_o$ alone produces calcium influx across the membrane, other ions being much less effective. This hypothesis is supported by observations that solutions having $10\times$ normal $[Mg]_o$ inhibited (presumably through competitive antagonism with calcium at membrane channels; Bolton, 1979) the photodynamic contraction almost as completely as did total $[Ca]_o$ removal. In apparent contrast to these results obtained at moderate light intensity, D600 or an increase in $[Mg]_o$ at high light intensity had much less effect on the photodynamic contraction although this could still be severely attenuated by omission of $[Ca]_o$ and completely blocked by the addition of EGTA to the calcium-free Krebs solution.

It seems therefore that at the lower light intensities the photosensitization effect involves a more persistant influx of Ca^{2+} through the Ca-channel and/or a depolarization which increases the discharge of Ca-spikes and evokes contraction (Braithwaite *et al.*, 1983). Both effects would be inhibited by D600 and an increase in $[Mg]_o$. At higher light intensities or upon prolonged exposure to illumination the photoactivation of erythrosine leads to a more extensive change in membrane permeability, primarily to Ca^{2+}, a large calcium influx occurs, and the force of photodynamic contraction becomes maximal; it cannot then be readily counteracted or offset unless EGTA is present. Under these conditions the muscle membrane must be rendered highly permeable to Ca^{2+} and the muscle is in effect photochemically 'skinned'. A similar explanation readily accounts for the progressive and parallel inhibition of the responses to carbachol and KCl seen in Figure 8b although here any 'skinning' action appears incomplete since lower intensity illumination was applied intermittently. The loss of sensitivity to agents which normally activate receptor- or voltage-operated channels, together with the development of tension dependent only on the ambient medium calcium, is characteristic of smooth muscles including taenia coli, that have been 'skinned' or 'stripped' by detergent action (Saida & Nonomura, 1978; Saida, 1982; Stout & Diecke, 1983). When compared to the extended time period for 'permeabilization' of the membrane by detergents, the action of erythrosine is virtually instantaneous (see Figures 2, 6 and 7) and is, moreover capable of precise photo-control by modulation of

the incident light intensity (Figure 3). Removal of the membrane permeability barrier by photochemical 'stripping' thus deserves further investigation since, when compared with other methods employing saponin, glycerol or ionic detergents, it provides a novel path for rapid access to the cytoplasmic compartment. Such a method is applicable not only to smooth muscle cells; the ability to control accurately $[Ca]_i$ after stripping the membrane photochemically has important implications for probing the stimulus-response coupling mechanisms of secretory, epithelial and myocardial cells.

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