Br. J. Pharmac. (1979), **66**, 197–201

CHARACTERIZATION OF ULTRAVIOLET LIGHT-INDUCED RELAXATION OF THE ISOLATED DUODENUM OF THE RAT

K.C. DAVE, M.N. JINDAL[1], V.V. KELKAR[2] & H.D. TRIVEDI

Department of Pharmacology, Smt. N.H.L. Municipal Medical College, Ahmedabad-380 006 and Department of Pharmacology, B.J. Medical College, Ahmedabad-380 016, India

1 Isolated duodenum of the rat, exposed to ultraviolet (u.v.) light in the presence of NO_2 ions, responded with reversible relaxation.

2 The photorelaxation response did not seem to involve any known receptor mechanisms and was independent of any ganglionic or neuronal influences.

3 Changes in the ionic environment of the tissue showed that Na^+ and Ca^{2+} were essential for the photorelaxation. K^+depolarized-tissue did not show the photoresponse.

4 The presence of the metabolic inhibitors, iodoacetic acid, 2,4-dinitrophenol, sodium fluoride, sodium azide or potassium cyanide, abolished the photorelaxation response.

5 It is proposed that the photorelaxation of the tissue resulted from the liberation of metabolic energy following NO_2 ion-dependent absorption of u.v. light energy, which in turn, interfered with the Na^+ ion movement across the cell membrane.

Introduction

Photosensitive reaction in a biological system is induced by the absorption of light energy by a substance of extraneous origin present in the tissue (Kirshbaum & Beerman, 1964). Many reports of photosensitive responses of smooth muscles are available in the literature. Kolm & Pick (1920) found light-induced stimulation of sensitized intestinal smooth muscles which was cholinergic in nature. Similar photostimulation responses of smooth muscles have been observed by other workers (Adler, 1919; Harris, 1925; Azuma & Hill, 1926; Azuma, 1927; Supniewski, 1927; Blum, 1941). Light-induced relaxation responses of smooth muscle preparations have also been reported. (Furchgott, Sleator, McCaman & Elchlepp, 1955; Furchgott, Ehrreich & Greenblatt, 1961; Ehrreich & Furchgott, 1968; Burnstock & Wong, 1978).

We have tested various isolated tissue preparations for their photoresponses in the presence of some known photosensitizers. It was observed that the isolated duodenum of the rat, when exposed to u.v. light in the presence of sodium nitrite, exhibited a consistent relaxation response (Trivedi, Kelkar, Jindal & Dave, 1978). In the present work, attempts have been made to characterize the photorelaxation response of this preparation. A possible mechanism leading to photorelaxation is discussed.

[1] Present address: Dean, M.P. Shah, Medical College, Jamnagar 361 001, India.

[2] Present address: Government Medical College, Surat, 395 007, India.

0007-1188/79/060197-05 $01.00

Methods

Pieces of duodenum from freshly killed albino rats were set up in a 10 ml organ bath or superfused by the technique described by Gaddum (1953). In some experiments, two anatomically adjacent pieces of duodenum obtained from the same animal were superfused in succession (cascade superfusion). Bathing medium in either case was Tyrode solution (composition, g/l: NaCl 8.0, KCl 0.2, $MgCl_2$ 0.1, $CaCl_2$ 0.2, NaH_2PO_4 0.05, $NaHCO_3$ 1.0 and glucose 1.0) kept at 31°C ± 1°C, and bubbled with O_2. All the experiments were conducted with the tissue kept in a dark chamber with a lid which permitted the exposure to u.v. light from a light source (Osram ultravitalux, Wotan, Germany) kept at a distance of 60 cm from the tissue. Unless stated otherwise, in all the experiments, the bathing medium contained sodium nitrite ($NaNO_2$), 0.5 g/l and the duration of exposure to the u.v. light was 30 s.

The responses of the tissue were recorded isotonically with a frontal writing lever (magnification, × 10; load, 0.5 g) on a smoked paper.

Reserpine-treated animals were prepared by the intraperitoneal injection of reserpine, 5 mg/kg, 24 h before they were killed.

Drugs

The following drugs were used: (−)-adrenaline bitartrate, acetylcholine chloride, 5-hydroxytryptamine

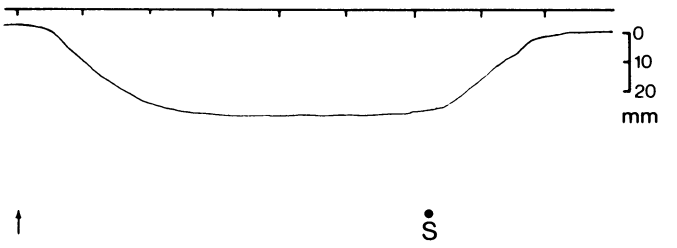

Figure 1 Kinetics of photorelaxation response of isolated duodenum of rat. The u.v. exposure began at (↑) and was terminated at (S). Time marks are at 5 s intervals.

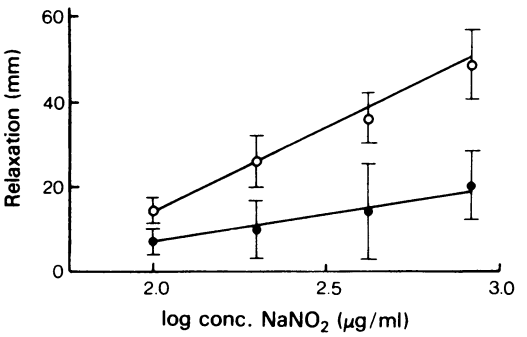

Figure 2 Photorelaxation response of rat isolated duodenum with varying concentrations of $NaNO_2$ in Tyrode solution. Tissues obtained from normal animals (○) and from reserpine-treated animals (●). Vertical bars show s.e. mean. Temperature 31 C ± 1°C and time of exposure 1 min.

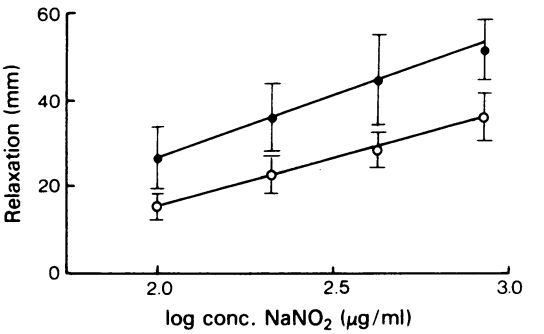

Figure 3 Photorelaxation response of rat isolated duodenum with varying concentrations of $NaNO_2$ in Tyrode solution and in the presence of morphine, 2 μg/ml, (●) and in the absence of morphine (○). Vertical bars show s.e. mean.

creatine sulphate, papaverine hydrochloride, tyramine hydrochloride, histamine acid phosphate, sodium nitrite ($NaNO_2$), potassium nitrite (KNO_2), physostigmine sulphate, morphine sulphate, phenoxybenzamine hydrochloride, atropine sulphate, mepyramine maleate, hexamethonium bromide, procaine hydrochloride, sodium azide, potassium cyanide, lithium chloride (LiCl) and strontium chloride ($SrCl_2$). The concentrations refer to the salts.

Solutions of nicotine, iodoacetic acid and 2,4-dinitrophenol were prepared fresh in Tyrode solution just before use.

A 1% solution of reserpine (Serpasil, Ciba) was used for injection.

Results

The tone of the rat isolated duodenum was not affected by either $NaNO_2$ or u.v. light exposure alone. However, the tissue responded with reversible relaxation when it was exposed to u.v. light in the presence of $NaNO_2$. Comparable photorelaxation was also obtained in the presence of KNO_2, 0.5 g/l.

The photorelaxation response showed a latent period of 1 to 2 s, reached its maximum in 10 to 15 s and persisted as long as the exposure continued. The recovery of the tone began immediately on termination of the exposure and was complete in 15 to 20 s (Figure 1). The magnitude of the response was linearly related to the concentration of $NaNO_2$, maximum response occurring at a concentration of 1 g/l although the response at this concentration was very variable and for this reason the data obtained at this concentration have been omitted from Figures 2 and 3. While involvement of any adrenergic mechanism was ruled out (see below), the photorelaxation was found to be reduced by prior reserpine-treatment of the animals (Figure 2). On the other hand, the presence of 2 μg/ml of morphine increased the photorelaxation of the tissue, shifting the dose-response curve of sodium nitrite to the left (Figure 3).

In the cascade superfusion experiments, exposure of the donor tissue alone to u.v. light did not affect the tone of the lower recipient tissue although both the preparations showed satisfactory relaxation when exposed to u.v. light separately (Figure 4).

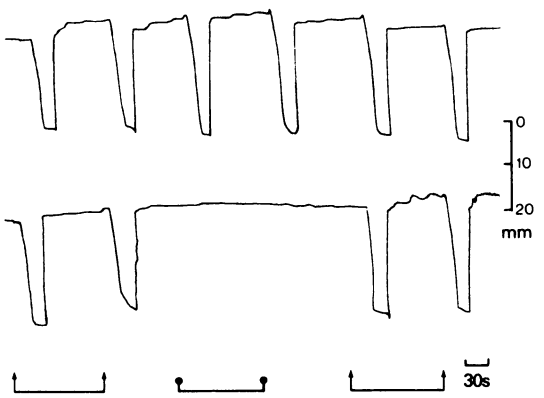

Figure 4 Effect of u.v. light in presence of $NaNO_2$, (0.5 g/l) on rat isolated duodenum in cascade superfusion experiment. The upper tracing shows responses of the donor tissue and the lower of the recipient tissue. Both the tissues were simultaneously exposed to u.v. light at (↑) while only the donor tissue was exposed at (●).

The photorelaxation response was not abolished by any of the receptor blocking drugs or pharmacologically active agents such as phenoxybenzamine, propranolol, atropine, morphine, mepyramine, hexamethonium, bretylium, cocaine or chloroquine (Table 1). The effective concentration of a particular receptor blocking agent was confirmed by blockade of the response of an appropriate agonist.

The photorelaxation as well as the adrenaline-induced relaxation of the tissue preparation were abolished by doubling the amount of NaCl or when NaCl was replaced by an equimolar concentration of LiCl in the Tyrode solution. The tissue, however, responded with relaxation to papaverine, 3 μg/ml. The K^+-depolarized tissue was relaxed on addition of adrenaline but the photorelaxation of the tissue was reduced to 2 to 5% of the normal response. Similarly, replacement of $CaCl_2$ by $SrCl_2$ in Tyrode solution abolished the photo-response without affecting the adrenaline response (Table 2).

The presence of metabolic inhibitors, iodoacetic acid, 2,4-dinitrophenol, sodium fluoride, sodium azide or potassium cyanide abolished the photorelaxation. The adrenaline response, was present with all the inhibitors except for iodoacetic acid (Table 3). The tone, whenever reduced by one of the metabolic inhibitors, was maintained by the addition of acetylcholine, 0.5 to 1 μg/ml. The photoresponse was restored to normal in all cases on washing the preparation with Tyrode solution.

Discussion

The reversible photorelaxation response of rat isolated duodenum to u.v. light, as observed in this study, confirms the findings reported by other workers (Ehrreich & Furchgott, 1968; Burnstock & Wong, 1978). However, the tissue preparation used here exhibited measurable and consistent photorelaxation responses at its natural level of tone. This obviated the use of a spasmogen to induce active tone in the tissue in order to obtain a good photorelaxation response as reported by some workers (Furchgott *et al.*, 1961; Ehrreich & Furchgott, 1968).

Table 1 Drugs that were unable to block the photorelaxation response of the rat isolated duodenum in Tyrode solution containing $NaNO_2$, (0.5 g/l)

	Concentration (μg/ml)	n	*Receptor blockade confirmed with* (μg/ml)	
Phenoxybenzamine	5	5	Noradrenaline	(2)
Phenoxybenzamine	5			
+ propranolol	10	7	Adrenaline	(2)
Atropine	1	4	Acetylcholine	(1)
Morphine*	4			
+ phenoxybenzamine	5	4	5-Hydroxytryptamine	(5)
Morphine*	4	5	—	
Mepyramine	10	4	Histamine	(3)
Hexamethonium	10	5	Nicotine	(1)
Bretylium	40	4	Tyramine	(10)
Cocaine**	10	3	—	
Chloroquine**	25	3	—	

* The photorelaxation response was augmented. ** The resting tone was reduced, but the photorelaxation response was still present.

The presence of a spasmogen is likely to interfere with characterization of the photoresponse and study of its mechanism. The photorelaxation was found to be proportional to the concentration of sodium nitrite from 0.1 g/l to 0.8 g/l.

Results of this study rule out the involvement of adrenoceptor, cholinoceptor, histamine or tryptamine receptor mechanisms in the photorelaxation. The response also appears to be independent of ganglionic or neuronal influences (Table 1). It is not possible to offer any explanation for the increased photorelaxation in the presence of morphine and its reduction in the tissues obtained from reserpine-treated animals. Further, the results of the cascade superfusion experiments negate the possibility of the release of any active substance from the tissue. On the other hand, the short latent period, the rapid development of the maximum response and the quick recovery of the tone on termination of exposure to u.v. light suggest that the photorelaxation may be the result of interference with the mechanisms primarily responsible for the maintenance of the tone of the tissue (Furchgott *et al.*, 1961).

It is known that relaxation can occur only in those muscles which maintain a tone (Bueding a Bülbring, 1964). Tone in visceral smooth muscle is normally maintained by a highly unstable membrane potential resulting from the high Na^+ permeability of the cell membrane (Kuriyama, 1963), and 95% of Na^+ in these tissues exchanges very rapidly (Goodford & Hermansen, 1961). This high Na^+ exchange may be because of the poor adsorption of Ca^{2+} at the membrane (Bueding & Bülbring, 1964).

With the above hypotheses in mind, it seems probable that there is liberation of metabolic energy following the nitrite-dependent absorption of light energy, promoting Ca^{2+} adsorption at the cell membrane and resulting in its stabilization and/or acceleration of active Na^+ extrusion. This proposal is consistent with the observations in this study that, (1) the

Table 2 Effects of changes in the ionic composition of the Tyrode solution on the u.v. light-induced relaxation and adrenaline-induced relaxation of the rat isolated duodenum

Ionic change in Tyrode solution, containing $NaNO_2$, (0.5 g/l)	n	*Photorelaxation*	*Adrenaline-induced relaxation*
NaCl replaced by equimolar conc. of LiCl.	7	Absent	Absent
2 × NaCl	7	Absent	Absent
2 × KCl	6	Feeble* or Absent	Present
$CaCl_2$ replaced by equimolar conc. of $SrCl_2$	7	Absent	Present

* The photoresponse, whenever present, was only about 2 to 5% of the photoresponse in normal Tyrode solution.

Table 3 Effects of various metabolic inhibitors on the u.v. light-induced relaxation and adrenaline-induced relaxation of the rat isolated duodenum in Tyrode solution containing $NaNO_2$, (0.5 g/l)

Metabolic inhibitor	*Conc. (μg/ml)*	n	*Photorelaxation*	*Adrenaline-induced relaxation*
Iodoacetic acid	100	5	Absent	Absent
2,4-Dinitrophenol*	150	6	Absent	Present
Sodium fluoride	100	5	Absent	Present
Sodium azide*	250	5	Absent	Present
Potassium cyanide*	100	5	Absent	Present

Adrenaline concentration was 3 μg/ml. * Slightly reduced tone restored and maintained by acetylcholine, 0.5 to 1 μg/ml.

photorelaxation response is dependent on the presence of both the NO_2 ions and the exposure to u.v. light; (2) photorelaxation is absent in K^+-depolarized tissue preparations which suggests that it results from changes in ionic fluxes at the membrane level; (3) the presence of Ca^{2+} is necessary for the photorelaxation response; (4) replacement of Na^+ by Li^+ in the bathing medium abolishes the response and (5) the presence of metabolic inhibitors abolishes photorelaxation.

The proposal is also consistent with the results of the electrophysiological study (Ehrreich, Kao & Furchgott, 1963) in which it was shown that during the photorelaxation of the smooth muscle the spike discharges are abolished and Na^+ permeability of the membrane is reduced.

We wish to thank Dr Miss Saudamini C. Pandya, the Dean, Smt. N.H.L. Municipal Medical College, for her keen interest and financial support for the work through the Ahmedabad Municipal Corporation, Mr S. B. Acharya for technical assistance and Mr C. P. Gami for secretarial assistance and for typing the manuscript. Reprint requests to K.C.D. at Smt. N.H.L. Municipal Medical College.

References

ADLER, L. (1919). Uber lichtwirkungen auf uberlebende glattmuskelige organe. *Naunyn Schmiedebergs Arch. exp. Path. Pharmak*, **85**, 152–162.

AZUMA, Y. (1927). Effect of Ca and K ions and ultraviolet rays upon involuntary muscle. *Proc. R. Soc.* B, **100**, 431–439.

AZUMA, Y. & HILL, L. (1926). Effect of ultraviolet radiation upon involuntary muscle and the supposed physiological interference of visible rays. *Proc. R. Soc. B*, **99**, 221–228.

BLUM, H.F. (1941) *Photodynamic Action and Diseases caused by Light*, p. 108 New York: Hafner Publishing Company.

BUEDING, E. & BÜLBRING, E. (1964). The inhibitory action of adrenaline. Biochemical and biophysical observations. In *Pharmacology of Smooth Muscle*. ed. Bülbring, E. p. 37, London: Pergamon Press.

BURNSTOCK, G. & WONG, H. (1978) Comparison of the effects of ultraviolet light and purinergic nerve stimulation on the guinea-pig taenia coli. *Br. J. Pharmac.*, **62**, 293–302.

EHRREICH, S.J., KAO, C.Y. & FURCHGOTT, R.F. (1963). Electrophysiological basis for photoactivated and drug induced relaxation in intestinal smooth muscle *Fedn Proc.* **22**, 309.

EHRREICH, S.H. & FURCHGOTT, R.F. (1968). Relaxation of mammalian smooth muscles by visible and ultraviolet radiation. *Nature, Lond.*, **218**, 682–684.

FURCHGOTT, R.F., EHRREICH, S.J. & GREENBLATT, E. (1961). The photoactivated relaxation of smooth muscle of rabbit aorta. *J. gen. Physiol.*, **44**, 499–519.

FURCHGOTT, R.F., SLEATOR, W.JR., MCCAMAN, M.W. & ELCHIEPP, J. (1955). Relaxation of arterial strips by light and the influence of drugs on this photodynamic effect. *J. Pharmac. exp. Ther.*, **113**, 22–23.

GADDUM, J.H. (1953). The technique of superfusion. *Br. J. Pharmac. Chemother.*, **8**, 321–325.

GOODFORD, P.J. & HERMANSEN, K. (1961). Sodium and potassium movement in the unstriated muscle of the guinea pig taenia coli. *J. Physiol.*, **158**, 426–448.

HARRIS, D.T. (1925). Studies on the biological action of light. *Proc. R. Soc. B*, **98**, 171–187.

KIRSHBAUM, B.A. & BEERMAN, H. (1964). Photosensitization due to drugs. *Am. J. Med. Sci.*, **248**, 445–468.

KOLM, R. & PICK, E.P. (1920). Uber beein flussung der automatischen tatigkeit des uberlebenden kalt and warmbluterdarmes durch fluoreszenzstrahlen. *Naunyn Schmiedebergs Arch. exp. Path. Pharmak*, **86**, 1–20.

KURIYAMA, H. (1963). The influence of potassium, sodium and chloride on the membrane potential of the smooth muscle of taenia coli. *J. Physiol.*, **166**, 15–28.

SUPNIEWSKI, J.V. (1927). The action of visible light in the haematoporphyrin sensitized organs. *J. Physiol.*, **64**, 30.

TRIVEDI, H.D., KELKAR, V.V., JINDAL, M.N. & DAVE, K.C. (1978). Some photoresponses of isolated tissue preparations to ultra violet light in presence of photosensitizers. *Ind. J. Physiol. Pharmac.*, **22**, 136–141.

(Received June 20, 1978.
Revised September 1, 1978.)