SOME PHOTORESPONSES OF ISOLATED TISSUE PREPARATIONS TO ULTRAVIOLET LIGHT IN THE PRESENCE OF PHOTOSENSITIZERS

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Summary: Responses of isolated tissue preparations to ultraviolet (UV) light were studied with and without the presence of photosensitizers like eosin, fluorescein and sodium nitrite. Exposure to UV light in the presence of sodium nitrite induced consistent relaxation of rat duodenum. The photo-relaxation was found to be related to the concentration of sodium nitrite. Adrenergic or cholinergic mechanisms do not seem to be involved. The isolated rat duodenum preparation exhibited quantitatively consistent photoresponse for 3 to 4 hr at its normal tone obviating the need for additional spasmogens as needed with other preparations. The preparation is a suitable test model for the study of photobiologic response evoked by UV light.

Key words: photoresponses *in vitro* UV light
sodium nitrite Rat duodenum

INTRODUCTION

The light induced photoresponses of animals and of isolated tissue preparations have been reported in the past. The photoresponses like stimulation of skeletal muscles (11,13,14,15), of heart (8,12) and of smooth muscles (1,9,10) have been observed. Recently, it was also reported that exposure to ultraviolet (UV) light induced relaxation in tonically contracted rabbit aortic strip(6,7). Similarly, other smooth muscles such as, circular strips of the corpus of the rabbit stomach, longitudinal strips from duodenum of rabbit, dog or cat, guinea pig taenia coli and strips from rabbit urinary bladder and uterus, kept in spasmogen-induced tonic state, showed relaxation when exposed to UV light in the presence of sodium nitrite (4).

The mechanism of such photobiological responses has not been thoroughly studied. Detailed pharmacological analysis requires a suitable test model. The tissue 'preparations used earlier are less suitable as they need spasmogen-induced tone to show the photore-

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laxation (4,6,7). The presence of spasmogen is likely to interfere with the study. The present work was undertaken to find a suitable *in vitro* test model.

MATERIALS AND METHODS

Skeletal muscle:

Rat phrenic nerve diaphragm preparation was set up by the method of Bulbring (2) using rats of either sex weighing between 200 to 250 g. For indirect stimulation, the nerve was stimulated with square wave pulses of 5 volts, 0.1 msec at 0.2 Hz. For direct stimulation, the muscle was stimulated with square wave pulses of 100 v, 1 msec at 0.2 Hz.

Rectus abdominis of frog was mounted in a simple organ bath by the method of Burn (3).

Heart muscle:

Isolated frog heart was perfused with frog-Ringer solution through a Syme's cannula tied into the inferior vena cava. The preparation was set up in a box which functioned as a dark chamber and an adjustable opening in the box allowed exposure of the preparation to UV light.

Isolated perfused heart of rabbit was set up by standard laboratory method (3).

Smooth muscle:

Aortic strip of rabbit (5), stomach strip of frog (16) and duodenum of rabbit and rat and ileum of guinea pig (3) were set up according to techniques described in the literature.

All the experiments were conducted in a semi-dark room prepared by keeping the windows closed and using thick curtains. Special dark chamber was, however, used for isolated perfused heart of frog as mentioned earlier.

The radiation energy was kept constant by using the same UV light source, namely a hand lamp of ultraviolet light (Osram, ultravitalux, Wotan, Germany), 300 watts, kept at 60 cm distance from the tissue. The responses in the experiments were recorded isotonically with a 10-fold magnification.

Drugs:

The following drugs were used:

Eosin, fluorescein, sodium nitrite, acetylcholine chloride, phenoxybenzamine hydrochloride, propranolol hydrochloride, and atropine sulphate. Their concentrations refer to the salts.

Eosin, fluorescein and sodium nitrite were included as photosenzitizers. Unless otherwise specified the concentration of eosin and fluorescein was 0.25 *mg/ml* while that of sodium nitrite was 0.5 *mg/ml*.

RESULTS

Skeletal muscle:

The skeletal muscle preparations were not affected by UV light exposure *per se*. The tissue preparations were also not affected by any of the photosensitizers in the absence of UV light.

UV light, in the presence of sodium nitrite, inhibited both directly and neuronally evoked contractions of rat phrenic nerve diaphragm preparation. The response was variable in different preparations and in the same preparations at different exposures. The inhibition of contractions was 24.6 ± 10.4 percent of the control response ($n=10$). It persisted as long as the exposure was continued. UV light in the presence of other photosensitizers did not show any effect.

ACh-induced contractures of frog rectus abdominis muscle were not affected by UV light in the presence of any of the photosensitizers used in this study ($n=3$).

Heart muscle:

The UV light exposure in the absence of a photosensitizer and the photosensitizers in the absence of UV light did not show any effect on the heart muscle preparations.

Frog heart, in the presence of sodium nitrite, responded to UV light showing reduction in the rate of contraction (in 5 out of 8 experiments) though the amplitude was not altered. The reduction in heart rate, was 43.2 ± 6.3 percent of the control rate ($n=5$). The photoresponse, whenever obtainable, was not affected by atropine (2 $\mu g/ml$), and persisted as long as the exposure continued. Normal heart rate was restored on termination of exposure to UV light. The other photosensitizing agents did not show such an effect.

Isolated mammalian hearts of rabbit ($n=5$), guineapig ($n=4$) and rat ($n=7$) were not affected by UV light in the pressence of any of the photosensitizers.

Smooth muscle:

None of the tissue preparations showed any response to UV light exposure in the absence of a photosensitizer.

The rabbit aortic strip showed no responses to UV light exposure in the presence of any of the three photosensitizers used in this study ($n=3$ each).

Eosin raised the tone of frog stomach strip whereas fluorescein or sodium nitrite had no effect. When the tissue was exposed to UV light in the presence of eosin it responded with relaxation. The response was slow to develop taking more than 40 sec for reaching maximum. The magnitude of relaxation was also feeble as compared to that of rat duodenum (vide infra).

Sodium nitrite but not the other two chemicals, sensitized the rat duodenum (n=20), guinea pig, ileum (n=4) and rabbit duodenum (n=6) to UV light producing relaxation. Sodium nitrite *per se* had no effect on any of these tissue preparations. The UV light-induced relaxation was quick to develop (1-2 sec) and the tissue tone returned to pre-exposure level on termination of the exposure.

The absence of pendular movement, and the easy availability of the animals made rat duodenum a suitable preparation for further study. It exhibited consistent photoresponse as the photorelaxation of the same magnitude was obtainable when the tissue was repeatedly exposed to UV light for 30 sec at intervals of 30 sec for 10-12 min (Fig. 1) or when exposed at intervals of 15 min for 4 hr. The photoresponse persisted as long as the exposure continued. The response was found to be related to the concentration of sodium nitrite between 100-800 $\mu g/ml$ (Fig. 2). It was unaltered in the presence of either phenoxybenzamine, (5 $\mu g/ml$), or propranolol (10 $\mu g/ml$) or both simultaneously (n = 3 each).

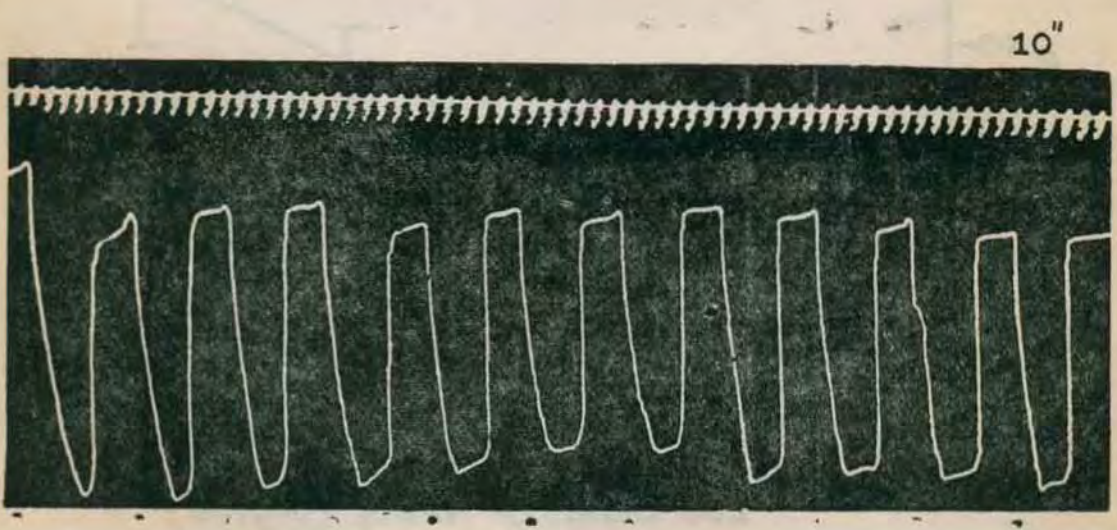

Fig. 1: Effect of repeated UV light exposures (at dots) on rat duodenum in presence of sodium nitrite (0.5 *mg/ml*). Exposure time was 30 sec with 30 sec interval between two consecutive exposures. Note the absence of tachyphylaxis.

DISCUSSION

The present study has shown that UV light exerts an inhibiting effect on rat phrenic nerve diapharagm preparation in the presence of sodium nitrite but has no effect on rectus abdominis of frog. These observations are not in agreement with the reported photostimulation of skeletal muscles wherein fluorsecent dyes were used as photosensitizers (11, 13, 14,15). The rate of frog heart decreased in the presence of UV light and sodium nitrite. Atropine in a concentration sufficient to block ACh effect did not alter this response suggesting that cholinergic mechanism was not responsible in the photoresponse of the heart muscle preparation. This observation differs from the reported cholinergic photostimulation of the chick-embryo heart (8).

Responses of smooth muscles to UV light in the presence of sodium nitrite or eosin differ from those reported by Kolm and Pick (10) but are in agreement with those of Furchgott *et al.* (4). Photoresponses exhibited by rat duodenum were consistent. The photoresponse was obtainable at normal level of tone of the tissue obviating the need for a spasmogen (4, 6). The presence of a spasmogen may complicate the pharmacolo-

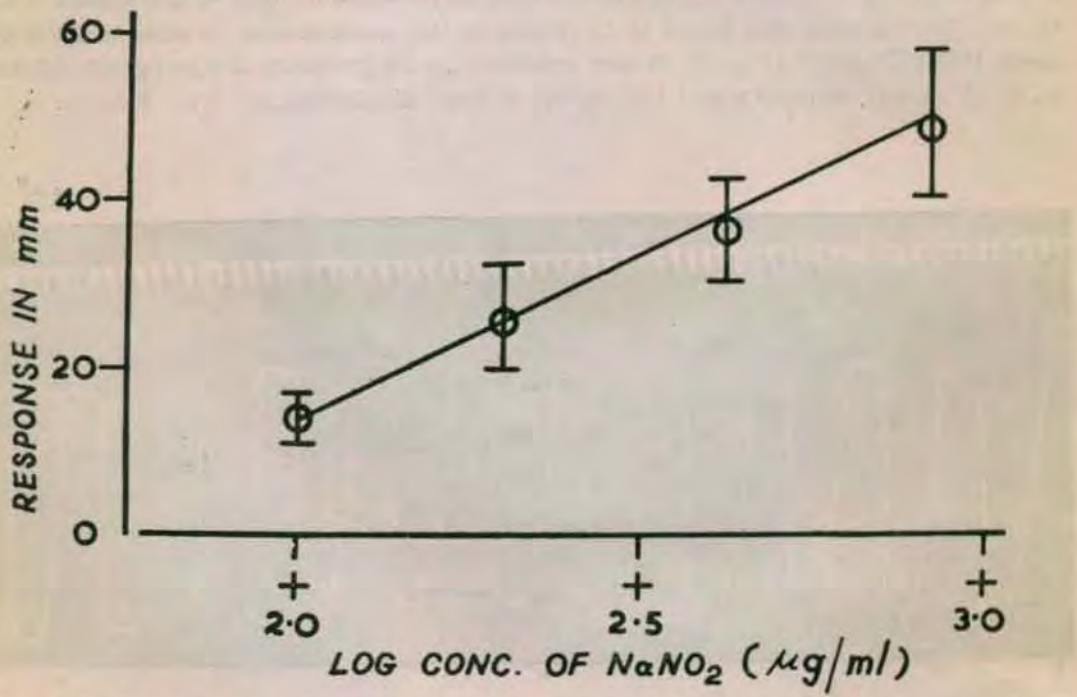

Fig. 2: Isolated rat duodenum. The dose-resopnse curve elicited by UV light exposures in presence of varying concentration of sodium nitrite. Each point is mean ±SE of 5 experiments. The tissue was not affected by these doses of sodium nitrite in dark or by UV exposure in absence of sodium nitrite.

gical analysis of photoresponses. Therefore, the consistent photoresponses in the absence of a spasmogen makes this preparation a suitable test model for detailed study. The photoresponse did not show any tachyphylaxis, and was independent of adrenergic receptor mechanisms as suggested by the inability of alpha and beta adrenergic receptor blocking drugs to alter it. The photoresponses were related to the concentration of sodium nitrite (Fig. 2).

The photoresponses obtained by other workers (4, 6, 7) required spasmogen-induced active tone. Rat duodenum, as observed in this study, could show photoresponses at its natural level of tone. The tissue also functioned for 3 to 4 hr without showing any signs of tissue damage exhibiting quantitatively consistent photoresponses. Grotthus-Draper law states that light must be absorbed in order to produce an effect. It is likely that the tissue, in the presence of sodium nitrite, absorbs the light energy provided by UV light exposures. The absorbed energy may be affecting ionic movement across the cell membrane finally resulting in the relaxation of tone of the tissue. If the function of a chemical sensitizer (or its combination with cellular organelle of the tissue) is to absorb light energy leading to a photoresponse in a biological system, it can be concluded that of the several test systems we studied, the rat duodenum with sodium nitrite as a photosensitizer, appears to constitute a suitable test-model for the study of photosensitization to UV light *in vitro*

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