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# LIGHT-ACTIVATED RELEASE OF NITRIC OXIDE FROM VASCULAR SMOOTH MUSCLE OF NORMOTENSIVE AND HYPERTENSIVE RATS

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*Summary.* A porphyrinic sensor was used to monitor nitric oxide release from vascular smooth muscle in response to exposure to ultraviolet light. Aortic rings exposed to UV light relaxed with a time course that parallels this observed NO release. With repeated UV light treatments, the magnitude of the relaxations diminished, suggesting that a store of NO was being exhausted. Photorelaxation in response to UV light was studied in aortic rings from two types of hypertensive rats, genetic (SHRSP) and nitroarginine-induced. These aortic rings showed greater photorelaxation and evidenced less tolerance than did aortic rings from control normotensive rats. Since NO synthase activity is depressed in both types of hypertension, it appears, paradoxically, that the UV light-releasable store of NO is augmented when NO synthase activity is depressed.
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Over thirty years ago Furchgott et al. (1) observed that ultraviolet light causes relaxation of vascular smooth muscle (VSM). Since that time extensive studies from Furchgott's laboratory (2-4) have established that the relaxation is independent of the endothelium, is accompanied by an increase in cGMP, and is potentiated by superoxide dismutase. These observations were interpreted as indicating that a release of NO by the UV light causes the relaxation.

The current study monitors directly, for the first time, the release of NO from VSM in response to UV light. These observations have permitted a measure of the time course of this release. No insight is evident as to the source of the NO, however, since NO release occurred within seconds, it did not appear to involve the induction of NO synthase, which requires hours. The current study also relates to our earlier observation (5) that NO synthase activity in VSM from hypertensive rats (SHRSP) is deficient compared to that of normotensive control rats (WKY). The current study compared VSM from these two strains of rats as to both photorelaxation and NO release in response to UV light.

## METHODS

### *Animals*

SHRSP and WKY rats were obtained from colonies in the Department of Anatomy and Cell Biology at the University of Michigan. These are inbred colonies derived from a stock supplied by the National Institutes of Health (NIH), Bethesda, MD.

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Rats were also made hypertensive by treatment with an arginine analog which blocks the action of NO synthase. For one week, WKY rats drank water with $N^{w}$-nitro-L-arginine (LNA) added to a concentration of 2.74 mM. Based on water consumption and animal weights, the average dosage of LNA for these animals was 52.6 mg/kg per 24 hours. Characteristics of the rats used in these studies are given in table 1.

*Aortic ring preparation*

Rats were anesthetized with pentobarbital sodium (50 mg/kg, ip). The thoracic aorta was removed and placed in physiological salt solution (PSS) of the following composition (mM): 130 NaCl, 4.7 KCl, 14.9 $NaHCO_3$, 1.18 $KH_2PO_4$, 5.5 Dextrose, 1.17 $MgSO_4.7H_2O$, 1.6 $CaCl_2.2H_2O$, and 0.03 $CaNa_2$ EDTA. The aorta was cleaned of loose connective tissue and cut into 4-5 mm rings. The rings were denuded of endothelium by gentle rubbing between the thumb and forefinger. Effectiveness of this procedure was confirmed for each ring by the observation that, when it was contracted with phenylephrine ($10^{-6}$M), it failed to relax in response to acetylcholine ($10^{-5}$M). Rings were mounted with 2g passive tension in a 40-ml water-jacketed organ chamber containing PSS at 37°C and aerated with 5% $CO_2$, 95% $O_2$. Contractions were measured with a Grass force transducer (FT03 Grass; Quincy, MA) and recorded on a Grass polygraph. After a 1 hr. equilibration period a concentration-response study with phenylephrine ($10^{-8}$, $10^{-7}$, $10^{-6}$, $10^{-5}$M) was performed.

*Ultraviolet light treatment*

The aortic ring was stimulated with the concentration of phenylephrine that gave a half-maximal response. Once the plateau of the contraction was established, the UV light was turned on for 10 minutes. These 10 minute light treatments were repeated at 30 minute intervals. UV lamp model UVGL-25 (Mineral Light) was used for these studies. A face of the lamp (power 4W, current 0.16A, UV emission at 366 nm) was positioned 6 cm from the aortic ring.

*Measurement of nitric oxide*

Measurements of nitric oxide were carried out with a porphyrinic microsensor. The NO sensor was prepared according to the procedure previously described (6,7). Polymeric film of nickel (II) tetrakis (3-methoxy-4-hydroxyphenyl) porphyrin was deposited on a single carbon fiber electrode (0.5-1.0 μm diameter) by cyclic scanning of potential between 0.2 to 1.0 V versus saturated colomel electrode, with scan rate of 100 mV/s. The sensor surface was coated with a Nafion film (5% w/w in alcohol). Differential pulse voltommetry or amperometry was used to monitor an analytical signal (voltommetric analyzer PAR263). The amperometric method (with a response time better than 10 ms) provides rapid quantitative response to minute changes of NO concentration. Differential pulse voltommetry, which also provides quantitative information but requires approximately 40s for the voltommogram to be recorded, was used mainly for qualitative analysis. Three-electrode system was used for the measurement of NO release. The three-electrode system consisted of a NO sensor working electrode, a platinum wire (0.25 mm) counterelectrode, and saturated colomel electrode reference electrode. The working electrode (L-shape electrode with carbon fiber mounted perpendicularly on glass capillary holder) was placed on the membrane of the single VSM cell of an aortic strip that had been denuded of its endothelium. NO concentration was determined from the measured current by means of a calibration curve and/or standard addition method. The porphyrinic sensor is not photoactive, i.e., irradiation of the sensor in buffer solution did not generate a photocurrent.

Table 1. Characteristics of rats used in photorelaxation studies

| | n | Wt. (gm) | BP (systolic, mmHg) |
|---|---|---|---|
| WKY | 4 | 260 ± 12.5 | 110.5 ± 5.7 |
| SHRSP | 4 | 218.5 ± 4.1 | 174 ± 5.9 |
| WKY (control) | 4 | 257.7 ± 10.0 | 134.7 ± 4.2 |
| WKY (LNA) | 4 | 274.5 ± 4.8 | 127.2 ± 1.7 (pre $R_X$)<br>152.0 ± 5.7 (post $R_X$) |

## RESULTS

*Tolerance of VSM to repeated UV light treatments*

Although the magnitude of the relaxation response to the initial dose of UV light was highly variable, it was consistently large, ranging from 40 to 95% of the phenylephrine contraction. When the identical 10 minute exposure to UV light was repeated 30 minutes later, the magnitude of the relaxation was much smaller. As is evident in figure 1, relaxations decremented asymtotically over the six UV light treatments at 30 minute intervals.

*Nitric oxide release in response to UV light*

Figure 2a depicts a typical amperogram (current-concentration versus time) showing NO release under illumination of the chopped (on-off) UV light from a denuded aorta strip of an SHRSP rat. An L-shaped porphyrinic sensor was placed on the surface of the single muscle cell in the strip. Five seconds after exposure of the strip to UV light, nitric oxide was detected by the sensor. The initial rate of NO release is 0.117 nM/s. This rate was changed to 0.030 nM/s after 60s of exposure to the light, and after about 450s nitric oxide concentration reached a plateau at 17.0 nM. A very similar profile of the aorta tension-time curve (figure 2b) was observed under identical experimental conditions to those applied for NO measurements. An initial decrease of tension with a rate of 5.8 mg/s is observed from the time of exposure to UV light up to about 60s. This decrease of the tension is 0.73± 0.03% per second (assuming 800 mg tension on plateau as 100%) which is within experimental error to that observed for the initial period of 60s for increase of NO concentration 0.69 ± 0.05% per second. After about 60s the rate of tension decrease

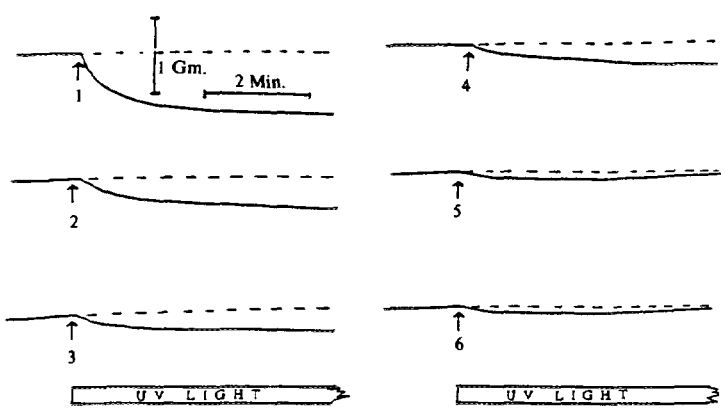



Figure 1. Tolerance to the relaxing effect of UV light. Aortic rings were made to contract with a concentration of phenylephrine that caused a half-maximal contraction. When the contraction had reached a plateau, treatment with UV light for ten minutes caused a relaxation. These ten-minute treatments were repeated six times at thirty-minute intervals. The magnitude of the relaxation decreased with each treatment. In between treatments the ring contracted back to the original plateau (record not shown).

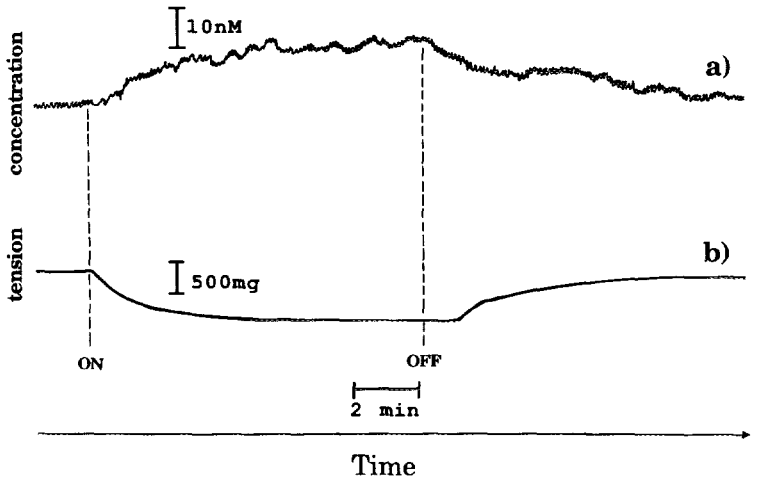



Figure 2. NO release and vascular smooth muscle relaxation in response to UV light treatment. Aortic wall, without endothelium, releases NO monitored with a porphyrinic sensor (a); and relaxes when treated with UV light for 10 minutes (b). Time courses of these two responses are parallel.

changed to 1.4 mg/s, i.e., 0.17% per second. Again this rate is similar to a rate of increase of NO concentration for that time interval (0.18% per second). The tension-time plot reached plateau 800 mg after about 350s of illumination with UV light. The illumination chopped off after 10 min. caused a linear decrease of NO concentration with a rate of 0.31 nM/s. However, about 30s delay of increase of tension was observed.

Consecutive illumination with UV light produced lower concentration of NO (15.0 nM and 11.5 nM after second and third illumination, respectively). It has been known that $O_2^-$ can be generated on photolysis of water by UV light (8). In the presence of 100 U/ml of SOD a maximum photolytic production of NO from aorta strip of SHRSP rats is established at a level of 27 nM which is about 59% higher than that of the concentration of NO produced in the absence of SOD.

Reaction of NO with $O_2^-$ is fast, diffusion controlled, with reported rate constant of $3.7 \times 10^7$ $M^{-1}$ $s^{-1}$ (9). Under conditions of simultaneous release of NO and $O_2^-$, the porphyrinic sensor (with response time of about a millisecond) is capable of detecting only unreacted portion of NO. Therefore, the 59% difference between NO production in the presence and absence of SOD can be accepted as consumed by $O_2^-$.

Amperometric curves showing NO release from aorta strips of normotensive rats (WKY) were also obtained. The profiles of these curves are similar to those described for hypertensive rats (SHRSP). However, an amount/concentration of NO produced is over 50% lower (6.6 nM) in comparison to that released from aorta strips of hypertensive rats.

*Photorelaxation of VSM from hypertensive rats*

The magnitude of photorelaxation of VSM from hypertensive rats was compared to this value of VSM from normotensive control animals. The extents of tolerance development to UV

light exposures in VSM from these two sources were also compared. These comparisons for VSM from SHRSP and from WKY are depicted in figure 3. It is evident that photorelaxation is greater in VSM from SHRSP and that the VSM from this source develops less tolerance to UV light treatment.

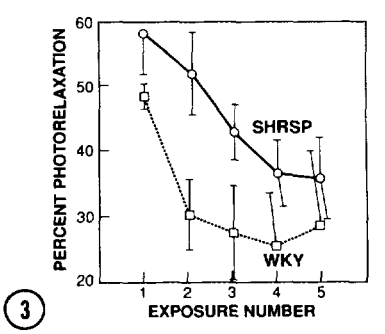
The same types of comparisons were made between VSM from WKY rats that had been made hypertensive by one week's treatment with LNA and VSM from normotensive, control WKY rats (figure 4). Again photorelaxation was greater and tolerance was less in VSM from the hypertensive rat.

## DISCUSSION

Although indirect evidence has given strong indications that UV light causes vascular smooth muscle relaxation by releasing NO in the tissue (2-4), the current study has furnished the first direct evidence of this action. NO was observed to be released from VSM in response to its irradiation with UV light. The time course of this release was observed in a parallel study to be identical to the time course of VSM relaxation, leaving no doubt that the interpretation of the earlier observations was correct.

Our observations that repeated treatments with UV light cause diminishing VSM relaxations and diminishing NO release indicates UV light causes the release of NO from a depletable store.

This observation invites speculation as to the nature of this NO store. Nitric oxide is known to react rapidly with molecules containing sulfhydryl groups (10). Because of this reaction molecules such as glutathione, cystine, or albumin may act as carriers or as biological sinks for NO (11). The kinetics of association and dissociation of the molecules, retaining or yielding NO, could be relevant to the storage of NO in VSM and its release in response to irradiation by UV light. Entirely parallel logic could be applied to the generation and dissociation of NO-heme

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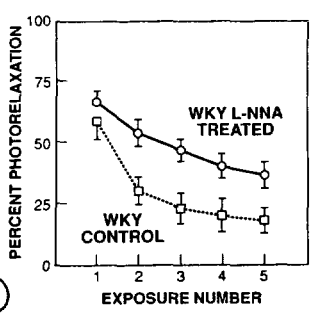


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Figure 3. Magnitudes of relaxation of aortic rings from WKY and from SHRSP. Data are presented as percent relaxation produced by a series of ten-minute exposures to UV light. The rings had been contracted with a half-maximal concentration of phenylephrine. Data are means ± SEM for four rats of each strain.

Figure 4. Relaxations of aortic rings from L-NNA-treated (hypertensive) and from control WKY rats (normotensive). Data are presented as percent relaxation produced by a series of ten-minute exposures to UV light. The rings had been contracted with a half-maximal concentration of phenylephrine. Data are means ± SEM for four rats in each group.

adducts (12). Also, nitrite which is a main product of NO oxidation can be photolytically decomposed with a regeneration of nitric oxide (3,4). Under similar experimental conditions used for study of smooth muscle photorelaxation, the porphyrinic sensor detected approximately 80 nM NO produced from 34 μM solution of $NaNO_2$ (pH 7.4). Therefore, it is very likely that nitrite accumulated in smooth muscle is one of the main sources of photolytic production of NO. Smooth muscle cells not in contact with endothelial cells do not show a production of NO after illumination with UV light. UV illumination of cultured smooth muscle cells from both WKY and SHRSP rats produced no concentration of NO detectable by the porphyrinic sensor.

The action of UV light was studied in two conditions known to be associated with a depressed NO synthase activity. Treatment with LNA is known to inhibit this enzyme and this action was evident in the hypertension that it produced after one week's treatment. The deficit in NO synthase activity and NO release results in an increase in vascular resistance that causes hypertension. We have recently observed that NO synthase activity is depressed in VSM cells cultured from SHRSP compared to those from WKY. It seems paradoxical that, as observed in the current study, in these conditions in which NO synthase activity is depressed there should be a greater storage of NO releasable by UV light irradiation. The interesting possibility is suggested that an increase in the storage of NO occurs as a physiological compensation for the decrease in NO production.

## ACKNOWLEDGMENTS

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