Depletion and Restoration of the Putative Photosensitive Materials Store Yielding Nitric Oxide in the Isolated Mouse Gastric Fundus

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ABSTRACT

We investigated the possibility of there being any photosensitive materials stores yielding nitric oxide (NO), and combined for the first time electrical field stimulation (EFS)- and UV light-induced relaxations in mouse gastric fundus. The tissue responded with relaxation to long wave UV light (366 nm). Repeated exposure to light decreased the fundic photorelaxation in that the initial photorelaxation was 31.5 ± 6.9% whereas the last (10th) photorelaxation was 2.3 ± 0.8%. There were no significant differences between EFS (30 V, 0.5 ms, 1 Hz, 15 s)-induced relaxations obtained before (39.7 ± 7.7%) and after (33.4 ± 9.1%) UV irradiation, which were completely blocked by 10⁻⁴ M L-N⁴-nitro-arginine methyl ester. Treatment of the tissue with NaNO₂, L-N⁴-nitro-arginine, S-nitrosoglutathione, or sodium nitroprusside for 30 min followed by prolonged washout restored the photorelaxation, whereas glyceryl trinitrate or L-arginine did not produce any improvement. EFS (30 V, 0.5 ms, 3 Hz) applied for 60 min significantly recovered the reduction of the photorelaxation, L-N⁴-iminoethyl-L-ornithine, which does not contain NO₂ moiety, abolished electrically induced relaxation; however, it did not change photorelaxations. UV irradiation caused relaxation only when the adventitial surface of the preparation was oriented to the source of UV light. These results indicate that there could be a photosensitive relaxant materials store yielding NO in the smooth muscle layer of the gastric fundus. This putative store can be refilled by NaNO₂, L-N⁴-nitro-arginine, sodium nitroprusside, S-nitrosoglutathione, or long-term EFS but not glyceryl trinitrate or L-arginine. Possible candidates for NO-yielding substances might not be an organic nitrate but an intracellular nitrite, nitrosylated substances, and unknown nitro-containing compounds, which could be all sensitive to UV light.

It has been known for many years that UV light induces relaxation in the rabbit aorta as first demonstrated by Furchgott et al. (1955). UV-elicited relaxation (photorelaxation), which is attributed to photo-induced relaxing factor, is independent of endothelium and is inhibited in that the initial photorelaxation was 31.5 ± 6.9% whereas the last (10th) photorelaxation was 2.3 ± 0.8%. There were no significant differences between EFS (30 V, 0.5 ms, 1 Hz, 15 s)-induced relaxations obtained before (39.7 ± 7.7%) and after (33.4 ± 9.1%) UV irradiation, which were completely blocked by 10⁻⁴ M L-N⁴-nitro-arginine methyl ester. Treatment of the tissue with NaNO₂, L-N⁴-nitro-arginine, S-nitrosoglutathione, or sodium nitroprusside for 30 min followed by prolonged washout restored the photorelaxation, whereas glyceryl trinitrate or L-arginine did not produce any improvement. EFS (30 V, 0.5 ms, 3 Hz) applied for 60 min significantly recovered the reduction of the photorelaxation, L-N⁴-iminoethyl-L-ornithine, which does not contain NO₂ moiety, abolished electrically induced relaxation; however, it did not change photorelaxations. UV irradiation caused relaxation only when the adventitial surface of the preparation was oriented to the source of UV light. These results indicate that there could be a photosensitive relaxant materials store yielding NO in the smooth muscle layer of the gastric fundus. This putative store can be refilled by NaNO₂, L-N⁴-nitro-arginine, sodium nitroprusside, S-nitrosoglutathione, or long-term EFS but not glyceryl trinitrate or L-arginine. Possible candidates for NO-yielding substances might not be an organic nitrate but an intracellular nitrite, nitrosylated substances, and unknown nitro-containing compounds, which could be all sensitive to UV light.
Materials and Methods

Tissue Preparation. Both sexes of mice (Mus musculus var albino) weighing 25 to 33 g were fasted overnight with free access to water. Mice were sacrificed by a blow to the head and exsanguinated. The stomach was carefully removed and the fundus was isolated. Approximately 10-mm-long and 2- to 3-mm-wide strips were prepared by longitudinal incision and oriented between two platinum wire electrodes with the adventitial surface toward the source of UV light under 0.5 g initial tension in an organ bath (5 ml) filled with Krebs' solution containing 5 × 10^{-4} M atropine. The organ bath maintained at 37°C was gassed with 5% CO₂ and 95% O₂. Responses were recorded on a polygraph paper (Gemini 7070; Ugo Basile, Varese, Italy) by isotonic transducers (7006, Ugo Basile). The composition of the Krebs’ solution was as follows: 118.5 mM NaCl, 1.2 mM KH₂PO₄, 4.8 mM KCl, 1.9 mM CaCl₂, 1.2 mM MgSO₄, 25 mM NaHCO₃, 10.1 mM glucose. The radiation source for photorelaxation was 6 W UV lamp with peak intensity at 366 nm (VL 6LC; Vilber Lourmat, Cedex, France). It was placed next to the outer wall of a jacketed glass incubation chamber. The distance between UV lamp surface and the preparation was about 2 to 3 cm.

Experimental Protocols. After the equilibration period of 1 h the tissue was submaximally preconstricted with KCl solution added to the organ bath to reach to a final concentration of 15 mM. After achieving a steady-state contraction the tissue was irradiated with UV light (366 nm) 10 times for 2 min at 3-min intervals (first series). It was then rinsed with Krebs’ solution and incubated for 1 h. Subsequently KCl was added to the bath to reconstitute the tissue. After preconstriction the tissue was irradiated using the same protocol as in the first series of experiments (second series). In some experiments electrical field stimulation (EFS, 30 V, 0.5 ms, 1 Hz) from a Grass stimulator (Grass Instrument Co., Quincy, MA) was delivered for 15 s both before and after UV light application. In another group of experiments after performing the first series of UV irradiations the tissue was rinsed with Krebs’ solution as mentioned above then incubated with 10^{-4} M NaNO₂, 10^{-3} M glycyl trinitrate (GTN), 5 × 10^{-5} M L-arginine nitro-arginine methyl ester (L-NAME), GSNO, and L-ARG (Sigma Chemical Co., St. Louis, MO). L-NIO was a gift from Dr. S. Cellek (The Wolfson Institute, London, UK). Except for L-NA all drugs were prepared as aqueous solutions. L-NA was dissolved in Krebs’ solution.

Analysis of Results. UV-elicited relaxations were expressed as percentages of KCl-induced contraction and shown as means ± S.E.M. Percent restoration of photorelaxations was calculated via the equation x / y × 100 = % Restoration.

Results

Photorelaxation. Isolated mouse gastric fundus responded with relaxation to UV irradiation (Fig. 1). There was a marked sensitivity difference to UV light among the tissues used through the study. Initial photorelaxation differed from one tissue to another in a range between about 10 and 80% of the KCl-induced tone, but usually around 20 to 35%. Consecutive application of UV light decreased the fundic relaxation from 31.5 ± 6.9% in the initial light stimulation to 2.3 ± 0.8% in the last UV stimulation (n = 10). In the second series, photorelaxation only recovered to 9.1 ± 1.9% in the initial response which was 30.4 ± 2.9% of the initial response obtained in the first series (Figs. 2 and 3).

When the mucosal surface of the gastric fundus was oriented to the source of UV light, slight relaxation was observed in response to UV irradiation; however, if the adventitial surface was directed to UV light it exhibited relaxation (n = 8, Table 1).

Effects of Repeated UV Light Application on EFS-Induced Relaxation. There were no significant differences between EFS-elicited relaxation obtained before (39.7 ± 7.7%) and after (33.4 ± 9.1%) UV light application (n = 10, Fig. 4). Electrically induced relaxations were completely blocked by 10^{-4} M L-NAME (n = 4, Fig. 4). Application of EFS before UV light did not affect photorelaxation (n = 6, Fig. 1B). In these series, photorelaxations from the first to the tenth were: 29.9 ± 5.3, 13.1 ± 2.7, 9.7 ± 1.8, 8 ± 1.8, 6.7 ± 1.6, 6 ± 1.5, 6.1 ± 1.5, 4.1 ± 0.7, 4.6 ± 1.2, and 3.9 ± 1.3% (not different from control).

Effects of NaNO₂, L-NA, L-ARG, SNP, GTN, and GSNO. Treatment of the tissue with 10^{-4} M NaNO₂ (n = 6), 5 × 10^{-5} M L-NA (n = 6; Fig. 3), 5 × 10^{-7} M SNP (n = 6), and 5 × 10^{-6} M GSNO (n = 6) for 30 min followed by abundant washout restored the photorelaxation (Fig. 5). Restoration values obtained after the treatment of NaNO₂, L-NA, SNP, and GSNO were 89.2 ± 8.1, 82 ± 17.2, 131.1 ± 30.2, and 89.1 ± 7.9%, respectively. However, 10^{-4} M GTN (27.9 ± 4.4%, n = 6) or 10^{-3} M L-ARG (25.7 ± 3%, n = 5) did not produce any restoration (Fig. 5). L-ARG (10^{-3} M) did not overcome the restorative effect of 5 × 10^{-5} L-NA. The restoration obtained after the treatment of L-ARG and L-NA was 129.8 ± 28.8% (Fig. 5, n = 7, different from control but not from L-NA group).

Influence of Long-Term EFS (30 V, 0.5 ms, 3 Hz, 1 h) on Photorelaxation. Stimulation (10-Hz) for 30 min did not affect restoration of the photorelaxation. The restoration obtained after EFS was 42.4 ± 6.2% (n = 8, not different from...
control). However, EFS (3 Hz) for 60 min produced a partial recovery of the photorelaxation that was significant (74.6 ± 11%, p < .025, n = 5; Fig. 6). In a separate group of experiments, 60-min incubation of 5 × 10^-4 M L-NA markedly blocked EFS-induced relaxation. Maximum relaxation decreased from 132 ± 35 to 15 ± 3.5% (p < .001, n = 7) in the presence of L-NA.

Effects of L-NIO on both EFS- and UV-Elicited Relaxation. L-NIO (10^-4 M) completely blocked electrically induced relaxation whereas it did not change photorelaxation. In the presence of this NOS inhibitor successive photorelaxations from the first to the tenth were: 29.5 ± 3.1, 12.3 ± 1.9, 10.3 ± 1.7, 9.7 ± 1.8, 8.1 ± 1.4, 6.4 ± 1.7, 6.1 ± 1.2, 5.2 ± 1, 4.6 ± 1.2, and 3.3 ± 0.8% (not different from control, Fig. 1C, n = 5).

Fig. 1. Photorelaxations of the isolated mouse gastric fundus on successive exposure to light (366 nm). Longitudinal strips were oriented between two platinum electrodes with the adventitial surface toward the source of UV light. Consecutive UV irradiation did not affect EFS (30 V, 0.5 ms, 1 Hz, 15 s)-induced relaxation, which was completely blocked by 10^-4 M L-NAME (A). Application of EFS before UV irradiation did not affect photorelaxation (B). L-NIO (10^-4 M for 15 min, n = 5), which does not contain NO2 moiety, abolished EFS (1 Hz)-induced relaxation whereas it had no effect on photorelaxation (C). Light applications are denoted by — and EFS by •. The breaks in the traces represent 15 min.

Fig. 2. Decline of photorelaxation on repeated UV light application in control (n = 10). Note slight recovery of the photorelaxation in the second series. Relaxations are expressed as percentages of 15 mM KCl-induced tone. Data represent mean ± S.E.M. of n observations.

Discussion

Exposure of rabbit aortic helical strips to light from a tungsten filament lamp causes relaxation (Furchgott et al., 1955). This photorelaxation, which was attributed to the photo-induced relaxing factor, has similarities to the relaxation by endothelium-derived relaxing factor (Furchgott et al., 1985, 1990). UV-evoked relaxation is inhibited by hemoglobin and methylene blue, and is accompanied by an increase in cGMP (Furchgott et al., 1985; Furchgott and Jothianandan, 1991). Similarly, in the isolated mouse gastric fundus long-wave (366 nm) UV light application produced relaxation, which was blocked by some agents that are known to inhibit NO-mediated responses such as hydroxocobalamin, FeSO4, methylene blue, and hemoglobin. In addition, it was potentiated in the presence of SOD (Oğulener et al., 1996). These findings provide evidence that NO or a related species is responsible for the photorelaxation in mouse gastric fundus. Ehrreich and Furchgott (1968) reported that the smooth muscle of the gastrointestinal tract, urinary bladder, and uterus appear to contain no endogenous photosensitive material or much less than does the smooth muscle of rabbit aorta. In the mouse gastric fundus, however, there does appear to be a photosensitive materials store because UV light produced relaxation in the present study. Venturini et al. (1993) demonstrated that photorelaxation
declined by approximately 60% on repeated exposure to light. These authors have hypothesized the existence of a photosensitive materials store in the rabbit aorta. Likewise, in the present study, consecutive application of UV light decreased the tissue relaxation approximately 90%. The fact that there is a sensitivity difference to UV irradiation from one tissue to another might suggest differing amounts of the preformed photosensitive materials store. Decline of the photorelaxation by repeated UV light exposure does not seem to be due to a desensitization of the tissue to NO because there were no differences between EFS-elicited relaxations obtained before and after UV light application. Furthermore, SNP-induced relaxation at the end of last photorelaxation was not changed.

### Table 1
The effect of orientation of the tissue on photorelaxations.

Two paired strips were prepared from the same tissue. Note the diminished photorelaxation when the mucosal surface of the tissue is toward the source of UV light. Photorelaxation was expressed as a percentage of KCl-induced tone (n = 8).

<table>
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<tr>
<th>Number of UV Light Applications</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucosal surface → UV</td>
<td>4.2 ± 1.8</td>
<td>1.3 ± 0.8</td>
<td>0.4 ± 0.4</td>
<td>0.2 ± 0.2</td>
<td>0.2 ± 0.2</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.1</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Adventitial surface → UV</td>
<td>25.5 ± 3.5</td>
<td>10.6 ± 2.3</td>
<td>5.5 ± 1</td>
<td>3.4 ± 0.6</td>
<td>3.9 ± 0.7</td>
<td>2.3 ± 0.5</td>
<td>1.9 ± 0.4</td>
<td>1.2 ± 0.6</td>
<td>1.1 ± 0.7</td>
<td>1.1 ± 0.8</td>
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Fig. 3. Representative tracings showing control photorelaxation in two series (A) and the restorative effect of L-NA (B, 5 × 10^{-5} M, n = 6). In control, after the last UV irradiation the tissue was washed with Krebs' solution and incubated for 1 h. Thereafter, a second series of UV irradiation was performed. In tracing (B) note the restoration of photorelaxation after the treatment of the depleted gastric fundus with L-NA. The tissue was in contact with L-NA for 30 min. Thereafter it was washed abundantly with fresh Krebs' solution and incubated for 30 min. The second series of UV irradiation was then performed. Light stimulation is denoted by —.

Fig. 4. Relaxations induced by EFS (30 V, 0.5 ms, 1 Hz, 15 s; n = 10) obtained before and after UV light application, and the effect of 10^{-4} M L-NAME (n = 4) on EFS-elicited relaxation. Relaxations are expressed as percentages of 15 mM KCl-induced tone. Data represent mean ± S.E.M. of n observations.

![Graph showing relaxation induced by EFS (Before UV, After UV, L-NAME)](image)

Fig. 5. Restoration of the photorelaxation after the incubation of several substances for 30 min followed by abundant washout. Control (n = 10), 5 × 10^{-5} M L-NAME (n = 6), 10^{-3} M L-ARG (n = 6), 10^{-6} M GTN (n = 6), 5 × 10^{-6} M GSNO (n = 6), 10^{-4} M NaNO₂ (n = 6), 10^{-3} M L-ARG + 5 × 10^{-5} M L-NAME (n = 7), and 5 × 10^{-7} M SNP (n = 6). *p < .025, **p < .01, ***p < .001, significantly different from control, Student's t-test for unpaired observation. Data represent mean ± S.E.M. of n observations.
by consecutive UV light applications (data not shown). It seems that UV irradiation does not activate NOS to produce NO because in the presence of L-NIO photorelaxation was not found to be different from control whereas EFS-elicted relaxation was abolished. The reason we have chosen L-NIO is that it does not contain NO₂ group (Moncada et al., 1997), which can be photolysed to release NO (Chang et al., 1993). The source of photosensitive materials seems to be within the smooth muscle layer of the preparation rather than mucosa as orientation of the tissue with the mucosal surface to UV lamp caused slight photorelaxations.

To manipulate the level of this store, we have made some attempts using NaNO₂, L-NA, SNP, GTN, GSNO, L-ARG, and long-term EFS. Although it was reported that exposure of the rabbit aortic strips to NaNO₂ did not restore photorelaxation (Venturini et al., 1993) we demonstrated that NaNO₂ significantly produced restoration. This difference may be due to the exposure duration and concentration of NaNO₂ used. It has been shown that photorelaxation is enhanced in the presence of nitrite ions (Ehrreich and Furchgott, 1968) but is not affected in the presence of SOD (Venturini et al., 1993; Charpie et al., 1994). However, when the bathing solution contain nitrite (NO₂⁻) ions, SOD potentiates photorelaxation (Matsunaga and Furchgott, 1989). In our previous study (Ögülener et al., 1996), however, SOD potentiated both photorelaxations in the absence or presence of NaNO₂, possibly indicating that mouse gastric fundus could contain nitrite, which may be one of the photoactivatable material sources. Generation of superoxide anions due to UV light has been reported (Matsunaga and Furchgott, 1989). If it is the case in this tissue they can be expected to react with NO to produce peroxynitrite, which then yields nitrate (Furchgott et al., 1990). Because a nitrate ion is not photoactivable (Furchgott, 1969) it is possible in our experimental condition that nitrate can be converted to NO₂⁻, which is photosensitive. It has been established that NO₂⁻ is decomposed to NO by UV irradiation (Matsunaga and Furchgott, 1989). On the other hand NO can be transformed into NO₂⁻ as an oxidation product in the cell. It has been reported that this transformation of NO into NO₂⁻ takes a long time, as much as hours (Butler et al., 1995). In the present study, after the resting duration of 1 h between the first and the second series in control, photorelaxation recovered about 30%, which is consistent with the report mentioned above. If the tissue contains an intracellular nitrite, its physiological and pathological importance has to be investigated.

SNP and GSNO produced restoration of the photorelaxation, indicating that a component of the photosensitive materials store could be nitrosylated compounds as iron sulfur complexes and nitrosothiols can be photolysed to release NO (Williams, 1985; Flitney et al., 1993). Moreover, it has been proposed that Fe-S nitrolys could act as cellular stores of NO (Butler et al., 1995). The fact that GTN could not produce any restoration in the photorelaxation is interesting, although it produced a profound relaxation (data not shown). This might reflect that it could produce relaxation via a different mechanism than the other relaxant substances used in the study. Matsunaga and Furchgott (1989) demonstrated that UV light (366 nm) did not release NO from GTN. Moreover, some other organic nitrates, such as pentaerythritol tetranitrate and mannitol hexanitrate, were found to antagonize photorelaxation. These findings along with the present data may show that an organic nitrate is not one of the sources of photosensitive materials. It has been reported that L-NA potentiates photorelaxation in rat trachea and aorta (Chang et al., 1993), rabbit aorta, pulmonary artery, and corpus cavernosum (Chen and Gillis, 1993) possibly because its NO₂ moiety liberates NO by UV irradiation. In the present study, L-NA treatment recovered the photorelaxation, possibly indicating that some nitro-containing compound(s) may be candidates for the photosensitive materials store. After treatment of L-NA the reduction in the photorelaxation on repeated UV application was observably smaller than that with the other chemicals. Coincubation with L-ARG failed to reverse its action. This suggests that the restoration is not NO synthase-dependent. An observatory finding is that the most sensitive tissues to be refilled appear to be those that exhibit relatively small initial relaxation to UV irradiation.

One of the most interesting facets of the present study is the filling up of photosensitive NO store by long-term EFS. The refilling effect probably resulted from NO release because prolonged incubation of L-NA markedly inhibited EFS-elicted relaxation. This is the first direct evidence that endogenous NO can replenish the photosensitive materials store. The physiological importance of this phenomenon seems worthy of being explored because it might point out a conservation mechanism for NO in the target (smooth muscle) cell. Further, it has been known that NO can react with some intracellular and membranal proteins containing thiol groups to form nitrosothiols, which can be photoactivable. In support of this, it has been reported that S-nitrosothiols could be the tissue source of NO-yielding photosensitive materials (Lovren and Triggle, 1998). The fundus of stomach actively dilates in response to low increases in intragastric pressure which now called “adaptive relaxation” accommodates the intake of liquid or food. NO has recently been reported to be responsible for this physiological response (Desai et al., 1991). NO also functions as an inhibitory neurotransmitter released by electrical stimulation in gastric fundus of several species (Boeckxstaens et al., 1991; Li and Rand, 1990; Barbier and Lefebvre, 1993; Büyükafşar et al., 1994; Lefebvre and Vandekerckhove, 1998). Taken together,
it seems plausible to postulate that NO released during food intake may replenish the photosensitive NO store.

It would be interesting to explore whether these photoactive substances could exhibit any biological rhythmicity, and any relationship between photosensitive materials and secretion of H⁺ from the gastric fundus.

In conclusion, according to the present study there could be a photosensitive compounds store yielding NO in the isolated mouse gastric fundus. The nature of photoactive materials appears to be complex. Among possible candidates for such photosensitive substances are at least not an organic nitrate but an intracellular nitrite that is an oxidation product of NO in the cell, nitrosylated compounds, and unknown nitro-containing substances that could be all sensitive to UV light.

References


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