A Photoactivable Source of Relaxing Factor in Genetic Hypertension

John R. Charpie, Anthony Peters, R. Clinton Webb

Abstract Deendothelialized rings of rabbit aorta relax after exposure to UV light because of release of a relaxing factor that is similar if not identical to nitric oxide. We tested the hypothesis that production of the photo-induced relaxing factor is impaired in a rat model of genetic hypertension. Thoracic aortas were removed from adult Wistar-Kyoto rats and stroke-prone spontaneously hypertensive rats. The vessels were cut into rings, denuded of endothelium, and placed in a muscle bath for isometric force measurement. Rings were contracted with phenylephrine, and relaxation was measured after exposure to UV light. Aortic rings from stroke-prone spontaneously hypertensive rats relaxed to a greater extent after exposure to UV light than did rings from Wistar-Kyoto rats. An inhibitor of nitric oxide synthase (N^6-nitro-1-arginine) greatly potentiated the relaxation responses to light in both strains, and these enhanced relaxations were attenuated by tetraethylammonium chloride, potassium chloride, ouabain, or inhibitors of guanylate cyclase. These results suggest that UV irradiation induces relaxation in aortic smooth muscle that is greater in hypertensive than normotensive rats and is greatly enhanced after addition of inhibitors of nitric oxide production. Thus, the unidentified photo-induced relaxing factor is not solely nitric oxide but may also represent either a hyperpolarizing factor, because depolarization blocks the responses entirely, or possibly smooth muscle guanylate cyclase that might itself be photoactivable. (Hypertension. 1994;23[part 2]:894–898.)

Key Words • ultraviolet rays • hypertension, genetic • nitric oxide • muscle, smooth, vascular • aorta

Previously it has been demonstrated that deendothelialized rings of rabbit aorta relax after exposure to UV light. This photorelaxation is reportedly independent of endothelium, is accompanied by a rise in cyclic GMP, is markedly potentiated by addition of nitrite ion or superoxide dismutase (SOD), and is inhibited by hemoglobin or methylene blue. These observations suggest that UV irradiation acts on some photosensitive material in the bathing solution and/or smooth muscle cell to produce a relaxing factor similar if not identical to nitric oxide (NO). More recently it has become apparent that there may be tissue and species differences in the release of photoactivatable substances from the vasculature and that results from different laboratories contradict the notion that UV light simply releases NO from vascular smooth muscle cells. We tested the hypothesis that production of a photo-induced relaxing factor is impaired in a rat model of genetic hypertension, and we sought to further characterize the properties of the relaxing factor in rat thoracic aortas.

Methods

The methods and procedures described in the present report were reviewed by the animal protocol review committee of the University of Michigan Medical School and are in accordance with institutional guidelines.

Aortic Tissue Preparation

Adult, age-matched female and male Wistar-Kyoto (WKY) rats (systolic blood pressure, 109±6 mm Hg; weight, 362±20 g; n=9; University of Michigan and Harlan Industries) and stroke-prone spontaneously hypertensive rats (SHRSP) (systolic blood pressure, 199±7 mm Hg; weight, 252±12 g; n=10; University of Michigan) were anesthetized with sodium pentobarbital (50 mg/kg IP) and exsanguinated. Thoracic aortas were removed and placed into cold physiological salt solution (PSS; mmol/L: NaCl 130, KCl 4.7, KH2PO4 1.18, MgSO4 1.17, CaCl2-2H2O 1.6, NaHCO3 14.9, dextrose 5.5, and CaNa2EDTA 0.03). The vessels were cleaned of adventitia and cut into 4-mm cylindrical segments under a dissecting microscope. The endothelium was removed from the arterial ring preparations by cannulating the lumen with microforceps and gently rolling the vessel between the forceps and palm. Previous histological work has demonstrated that this rubbing procedure removes at least 95% of the endothelium. In all experiments (see below) the absence of endothelium was confirmed by no relaxation to acetylcholine (10^-7 mol/L) after contraction induced by a concentration of phenylephrine that produced a near half-maximal response (EC50).

Muscle Bath Experiments

The vessels were transferred to warm PSS (37°C) and mounted in 50-mL jacketed organ baths for measurement of contractile activity. Vessels were bubbled with 95% O2 and 5% CO2 throughout the experiment. The vessel lumen was threaded with two wires fastened to two stainless steel support blocks. One block was mounted on a force transducer (Grass FT.03) and the other on a displacement device so that passive force (3 g) could be applied. After preparation, the vessel segment was allowed to equilibrate for 90 minutes in PSS. Contractile force was measured (in grams) after exposure of the vascular segments to increasing concentrations of an agonist.

Ultraviolet Light Experiments

Relaxation responses were measured after exposure of arterial rings to long-wave (366 nm) UV light (Mineralight UV SL 25). The UV lamp was mounted next to the 50-mL jacketed...
were inversely correlated with the force of the preceding relaxations in response to UV light was greater for aortic rings from both Wistar-Kyoto (WKY) rats (top tracing) and stroke-prone spontaneously hypertensive rats (SHRSP) (bottom tracing). The magnitude of photorelaxation was significantly greater in SHRSP than WKY rats before addition of phenylephrine and at the lowest agonist concentrations.

Before addition of phenylephrine, SHRSP but not WKY agonist-induced contractions for both rat strains (Fig 1). Furthermore, at phenylephrine concentrations (SHRSP versus WKY rats) (open squares) at 10⁻³ mol/L phenylephrine (P<.05). At phenylephrine concentrations greater than 10⁻³ mol/L, relaxation responses to UV light were not significantly different between WKY and SHRSP rings. N⁵-nitro-L-arginine (L-NNA) treatment of both WKY (open circles) and SHRSP (filled circles) aortic rings significantly enhanced photorelaxation responses at all phenylephrine concentrations (P<.05). In addition, L-NNA treatment significantly increased photorelaxation responses in SHRSP more than in WKY rings (P<.05). Values are mean±SEM. *Statistically significant difference between SHRSP and WKY rings.

To determine whether the photorelaxation responses were due to production of a relaxing factor in the aortic smooth muscle or within the bathing solution, we placed a black screen between the UV lamp and aortic ring preparations (but not between the lamp and buffer solution). Placement of the screen abolished the photo-
Despite the presence of inhibitors of vasodilatation. After an EC50 concentration of phenylephrine, relaxation to UV light (control) was not different between aortic rings from Wistar-Kyoto (WKY) rats and stroke-prone spontaneously hypertensive rats (SHRSP). Nω-nitro-L-arginine (+L-NNA) treatment significantly increased photorelaxation in SHRSP more than WKY rings (P<.05). Subsequent incubation with 40 mmol/L potassium chloride (+KCl), 20 mmol/L tetraethylammonium chloride (+TEA), or 1 mmol/L ouabain (+ouabain) significantly attenuated light-induced relaxation in both WKY and SHRSP rings. Treatment with LY83583 (+LY83583), a guanylate cyclase inhibitor, abolished photorelaxation in both WKY and SHRSP rings. Nitroglycerin (10 μmol/L) caused complete relaxation in both WKY and SHRSP rings, with no significant difference in response. Values are mean±SEM. *Statistically significant difference between SHRSP and WKY rings.

relaxation responses, and removal of the screen restored the relaxation responses.

Discussion

UV light–induced relaxation was first described in 1955 by Furchgott8 in isolated strips of rabbit thoracic aorta. Subsequently, in vitro experiments with a perfusion superfusion system allowed Furchgott and coworkers9,10 to conclude that UV irradiation acts on some photosensitive material in the smooth muscle membrane to produce a labile factor similar to endothelium-derived relaxing factor (EDRF). With the identification of EDRF as NO in 1988, it was hypothesized that the photo-induced relaxing factor was probably NO. Given this background, we proposed that UV light might provide a nonpharmacologic tool for studying relaxation mechanisms of vascular myocytes in hypertension. Results from the present study show that SHRSP aortic rings have a significantly greater relaxation response to UV light at baseline and after low-dose agonist-induced contraction than those from WKY rats. Furthermore, addition of L-NNA greatly potentiates these photorelaxations and to a significantly greater extent in SHRSP than WKY rings. Depolarizing agents and guanylate cyclase inhibitors attenuate the UV light–induced relaxations.

Chen and Gillis3 recently described a similar enhancement of photorelaxation in rabbit aorta, pulmonary artery, and corpus cavernosum incubated with L-NNA before UV irradiation. The enhanced photorelaxation was accompanied by increased cyclic GMP levels. These researchers hypothesized that the enhanced relaxation was due to conversion by UV light of the NO2 group of L-NNA to a nitrosyl radical, with resultant activation of guanylate cyclase. To address the possibility that enhanced photorelaxation in rat thoracic aortas was due to the release of NO2 from L-NNA, we exposed the L-NNA stock solution to UV light for 15 to 20 minutes before its addition to the muscle bath. In contrast to what one would expect if UV light released nitrosyl radicals into the muscle bath, we observed no relaxation. Second, if photo-induced relaxation depended on release of NO2 from L-NNA, then this response probably would not be reproducible and reversible unless L-NNA was in vast excess or the excess nitrosyl radicals were immediately scavenged (by superoxide, for example) after the light was turned off. If this were the case, one would expect enhancement of relaxation after addition of SOD, which inhibits superoxide formation. However, we observed no such enhancement after SOD. Furthermore, nitroglycerin produced complete relaxation in both WKY and SHRSP rings (suggesting a similar responsiveness to NO donors), yet L-NNA enhanced photorelaxation more in SHRSP than WKY rings. Karlson et al10 showed enhancement of photorelaxation and cyclic GMP production in bovine mesenteric arteries made tolerant to nitroglycerin, suggesting a different mechanism of relaxation between light and nitroglycerin. Finally, to test whether the photo-induced relaxing factor was released by the smooth muscle in response to UV light, we placed a physical barrier between the light and aortic ring (but not between the light and buffer). Placement of the barrier abolished the relaxation to UV light, and removal of the barrier restored the response entirely. Chen and Gillis3 demonstrated increased photorelaxation after treatment with the dihydropyridine derivative BAY K 8644 (which does contain an NO2 group but is not known to stimulate NO release) and observed no increased potentiation of photorelaxation after SOD, as expected if the relaxation to light were due to NO.

Earlier studies have suggested that there is an endothelium-dependent deficit of vasorelaxation in genetic hypertension,1114 and endothelium-independent relaxation responses (to papaverine or nitroprusside, for example) remain intact compared with normotensive control rats. In contrast with these pharmacologic studies, our findings suggest that UV light–induced relaxations are enhanced in hypertension, thus rejecting our original hypothesis. An important consideration in the interpretation of our results is that before addition of the contractile agonist, UV light induces a large relaxation in aortic rings from SHRSP but not WKY rats. This implies that there is significant basal myogenic tone in SHRSP compared with WKY rings. Our results clearly show a strong interdependence between the degree of photorelaxation and the magnitude of prior contraction for both rat strains. Thus, the initial increased basal tone in SHRSP affects the magnitude of relaxation at each phenylephrine concentration. When one examines the percent relaxation after an EC50 of phenylephrine, the results appear more similar between SHRSP and WKY rings (see Figs 2 and 3). This increased basal resting myogenic tone in SHRSP aortic rings may partly explain the apparent abnormal relaxation responses to pharmacologic agents observed in certain animal models of genetic hypertension.

An additional explanation for the differences in relaxation responses to UV light and NO donors is that the photo-induced relaxing factor may not be NO.
Wigullis et al.15 studied the effects of NaNO2 on UV light-induced relaxation and guanylate cyclase activation in bovine mesenteric arteries. These researchers found that in general the arteries reversibly relax to UV light; however, some strips were insensitive or even responded with a small contraction. The basal guanylate cyclase activity in the absence of NaNO2 did not increase after UV light exposure despite decreased tone in many vessels. Addition of NaNO2 potentiated the relaxation in all strips and markedly increased guanylate cyclase activity. Thus, the mechanism of relaxation to light may be different before and after NaNO2 administration. Raffa et al.16 found a critical dependence of photorelaxation on extracellular sodium concentrations similar to acetylcholine-induced relaxation in rabbit thoracic aorta. In contrast, however, the responses to acetylcholine and UV light were fundamentally different in their sensitivities to substitution of sodium with lithium or choline, suggesting a different mechanism of relaxation other than NO-induced activation of guanylate cyclase. Furthermore, photorelaxation has been found to be enhanced by NaNO2,17 with further potentiation of these responses by SOD. These results suggest that part of the enhanced response in the presence of NaNO2 is due to photoactivation of nitrite to generate a labile relaxant (that is probably NO) and in the absence of SOD is inactivated by O2·− in solution. In the absence of NaNO2, however, SOD does not significantly enhance photorelaxation.18 Thus, photo-induced relaxation is probably not entirely due to the generation of NO in the absence of exogenous nitrite administration. Our results would agree with this hypothesis. Deendothelialized rat thoracic aortas relaxed in response to UV light, and incubation with L-NNA, an inhibitor of NO synthase, greatly potentiates photorelaxation in both WKY and SHRSP aortic rings, suggesting that photorelaxation is not due to the release of NO from the smooth muscle. Addition of SOD had little or no effect on the photorelaxation response.

To further investigate the mechanism of photorelaxation, we tested other inhibitors of the NO-guanylate cyclase pathway. High-dose methylene blue and LY83853 attenuated the photorelaxation responses. Photorelaxation was inhibited by 40 mmol/L KC1, 1 mmol/L ouabain, or 20 mmol/L TEA in both WKY and SHRSP rings out of proportion to the increased tone produced by these agonists. These data suggest that at least part of the enhanced relaxation response in the presence of L-NNA is due to cellular hyperpolarization rather than NO release from the smooth muscle. This would be consistent with release of a photo-induced relaxing factor from smooth muscle that is similar to endothelium-derived hyperpolarizing factor (EDHF).19 EDHF is an as yet unidentified factor distinct from NO that is inhibited by ouabain and other agents that depolarize the cell.20 EDHF acts by opening K+ channels in the membrane, thus opposing myocyte contraction and promoting relaxation. Indomethacin had no significant effect on photorelaxation, suggesting that prostacyclin plays a small role in the response to UV light. Karlsson et al.21 measured intracellular cyclic AMP levels in bovine mesenteric arteries and observed no increase after UV light exposure. These results were also confirmed in aortas from rats.21

An additional tenable hypothesis is that guanylate cyclase is directly photoactivated to stimulate increased cyclic GMP production and vasodilation. Karlsson et al.19 showed that the cyclic GMP—including effect of UV light was probably due to a direct influence on guanylate cyclase activity. These authors demonstrated an increased maximal velocity and Km for guanylate cyclase after UV light exposure that are consistent with the effects of other activators of the enzyme.

In summary, precontracted rings of rat thoracic aorta relax in response to UV light, and the photorelaxation response is greater in rings from genetically hypertensive rats than normotensive rats. Furthermore, both rat strains demonstrate an enhanced relaxation response after L-NNA treatment, and this enhanced response is greater in SHRSP than WKY rats at all phenylephrine concentrations tested. Pharmacologic agents that depolarize vascular myocytes significantly attenuate the enhanced photorelaxation after L-NNA, suggesting that NO is not likely to be the photo-induced relaxing factor, but it may be more similar to the as yet unidentified EDHF. Another possibility is that smooth muscle guanylate cyclase is directly activated by UV light to cause vasodilation.

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References


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