

Mechanism of UV light-induced photorelaxation in isolated rat aorta

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Isolated rat thoracic aorta which is pharmacologically precontracted by phenylephrine induces photorelaxation when exposed to long wave length UV-light. The aim of the present study was to characterize the mechanism of UV-light induced by photorelaxation in the rat aorta. 1. UV light relaxed both endothelium-intact and -denuded rat aortic rings contracted by phenylephrine. The magnitude of relaxation on UV light was dependent on the exposure time and slightly greatly in endothelium-denuded rings than in endothelium-intact preparations. 2. L-NAME (10 nM-100 μ M) but not D-NAME completely inhibited the photorelaxation in a concentration dependent manner. 3. The UV-induced relaxation was inhibited by methylene blue (1–100 μ M), and verapamil (100 nM), and removal of extracellular Ca^{2+} . In contrast, UV-light induced photorelaxation was potentiated by N^{ω} -nitro-L-arginine (L-NOARG) treatment. 4. In immunocytochemical analysis of UV-light induced iNOS and eNOS expression in rat aortas, at which expression levels were increased in a time-dependent manner on UV-irradiation in aortic endothelium and smooth muscle, respectively. These results suggest that UV light-induced photorelaxation may be due to nitric oxide from exogenously administered L-arginine as well as endogenous nitric oxide donors such as amino acid and arginine derivatives. Additional suggestion is that UV light stimulates the expression of nitric oxide synthases, and its activity for nitric oxide generation is dependent on cytosolic Ca^{2+} originated from extracellular space.

Key words: Photorelaxation, endothelium, EDRF, rat aortic artery, nitric oxide synthase, nitric oxide

Introduction

In 1980, Robert Furchgott demonstrated the role of endothelial cells during the relaxation of isolated rabbit

aorta exposed to acetylcholine (1). This seminal observation has become crucial to the understanding of the regulation of vascular smooth muscle tone. His simple pharmacological experiment has initiated numerous studies on a wide variety of blood vessels, and has lead to the understanding of a new physiological role for nitric oxide. Also, nitric oxide is implicated in the pathogenesis of cardiac failure. There is now considerable evidence that nitric oxide plays a role in regulating myocardial function [2, 3, 4].

Over two decades ago, several investigators reported that the isolated tissues which were pharmacologically contracted relaxed when exposed to UV light [5, 6, 7, 8, 9]. In these reports, changes in the ionic environment of the tissue found that Na^{+} and Ca^{2+} were essential for this photorelaxation [6] which proved reversible [6, 7, 9]. Recently, photorelaxation of arteries by UV light is hypothesized to result from nitric oxide (NO) released from photoactivable stores [10]. Also, a study reported enhanced photorelaxation of aortic tissue from rats administered the NO synthase (NOS) inhibitor N^{ω} -nitro-L-arginine (L-NNA) [11]. Presumably, this potentiated photorelaxation was due to NO generated from the UV light-induced decomposition of the NO_2 moiety of L-NNA.

Nitrite is a stable end product of nitric oxide metabolism. In fasted individuals as much as 90% of circulating nitrite is derived from the L-arginine nitric oxide pathway and is a valid indicator of nitric oxide production [12]. Although nitric oxide appears to be the major vasodilator released by endothelial cells in a vast majority of blood vessels, other substances, some of them still unknown, may also play a role [1, 13, 14].

Previously, we have been investigated the roles of nonadrenergic, noncholinergic (NANC) nerve fibers which may act on NANC nerve transmitter substances. Among the substances of putative NANC neurotransmitters, purine nucleotides are considered as the most likely candidate for NANC neurotransmitter [15, 16, 17]. However, whether NO is also one of the NANC members has not been studied yet. Thus, in this study we examined

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the role of NOS isoforms and Ca^{2+} ion at the rat aorta.

Materials and Methods

Materials

The following chemicals were used: Phenylephrine HCl (PE), acetylcholine chloride (Ach), N^{ω} -nitro-L-arginine methyl ester (L-NAME), N^{ω} -nitro-D-arginine methyl ester (D-NAME), methylene blue (MB), L-arginine (L-Arg), and verapamil. These chemicals were purchased from the Sigma Chemical Co. Other chemicals used were of analytical grade.

Animals

The rats (Sprague-Dawley) used in this study, either sex, weighing 200-250 g, were killed by decapitation and exsanguinated. Rats were housed in an air-conditioned, light- and temperature-controlled environment. Throughout this study, rats were fed and watered *ad libitum*.

Tissue preparation

The aortas from the exsanguinated rats were removed, the arteries cut into rings (approximately 3-4 mm length) and the surrounding fat and connective tissue removed in 4°C ice-cold Krebs ringer solution, of the following composition (mM): NaCl, 120; KCl, 4.75; Glucose, 6.4; NaHCO_3 , 25; KH_2PO_4 , 1.2; MgSO_4 , 1.2; and CaCl_2 , 1.7 (mM, pH 7.4), and used for organ bath studies.

Recording system

The rings were suspended horizontally between two parallel platinum wire electrodes, the lower end was fixed at basement of a water-jacketed organ bath (volume 10 ml), and the upper end was attached to a transducer. The bath medium was maintained at $37 \pm 0.5^\circ\text{C}$ and gassed with 5% CO_2 in O_2 . Changes in the aortic preparation tension were recorded by an isometric force transducer (FT03) and ink-writing curvilinear polygraph (79, Grass) [15, 16, 17].

UV-light Photorelaxation

At the beginning of the experiments, the preparations were allowed to equilibrate at a 1 g resting tension for 60 min prior to chemical administration. To allow studies of the photorelaxation, each strip was precontracted by 1 μM phenylephrine (PE). After a plateau was reached, the aortic strip was exposed to UV-light (366 nm wave-length) for indicated time. This UV-light application was repeated three times at 3-min intervals. The aortic strip was then rinsed with Ca^{2+} -containing or -free Krebs ringer solution and allowed to rest for 30 min. After incubation, the same procedure was repeatedly applied on the same preparation. The UV lamp was mounted next to the outer wall of the water-jacketed organ bath, and the distance from the lamp to the preparation during irradiation was about 3-4 cm [5,

7, 8].

Immunohistochemistry

Immunohistochemical detection of iNOS and eNOS was performed as described previously [18, 19]. Briefly, another prepared aorta in the same condition as that used in the organ chamber study was fixed using 4% paraformaldehyde and incubated with monoclonal anti-iNOS, and anti-eNOS primary antibodies (1 : 100) diluted in phosphate-buffered saline containing 1 mg/ml bovine serum albumin for 2 hr, rinsed with the same solution for 30 min, and incubated with biotinylated goat anti-mouse IgG (1 : 200) for 60 min. The samples were then exposed to avidin-biotin complex and reacted with DAB according to the manufacturers recommendations and counterstained with hematoxylin.

Results

Effects on endothelium of UV light- and Ach-induced relaxation

Ach-induced relaxation was completely diminished in endothelium-denuded aorta (Figure 1, upper panel). UV light induced time-dependent relaxation in both endothelium-intact and -denuded aortas contracted with phenylaphrine (PE). UV light-induced relaxation was independent of endothelium. But, the potentiation of relaxation of was significantly greater in endothelium denuded than endothelium intact aorta (Lower panel of Figure 1 and Figure 2).

Effects of L-arginine and NOS inhibitor on UV light-induced photorelaxation

Rat aortas precontracted with 1 μM PE showed time-dependent relaxation of UV light exposure. Increased vessel tone was significantly diminished with nitric oxide synthase inhibitor, L-NAME (Figure 3A) but not with derivative, D-NAME (Figure 3B). The magnitude of the photorelaxation was slightly increased with D-NAME (Figure 3B). Moreover, the developed tone gradually depressed upon L-arginine administration and then showed that the augmented photorelaxation by L-arginine was also dependent on the exposure time to UV light (Figure 3C).

Inhibitory effect of methylene blue (MB) on UV light-induced photorelaxation

To identify the interrelation of cGMP and photorelaxants derived from UV light-induced photorelaxation, soluble guanylyl cyclase inhibitor, methylene blue was introduced in rat aorta. Figure 4 shows that methylene blue inhibits the potentiation of UV light-induced photorelaxation, in a concentration dependent manner.

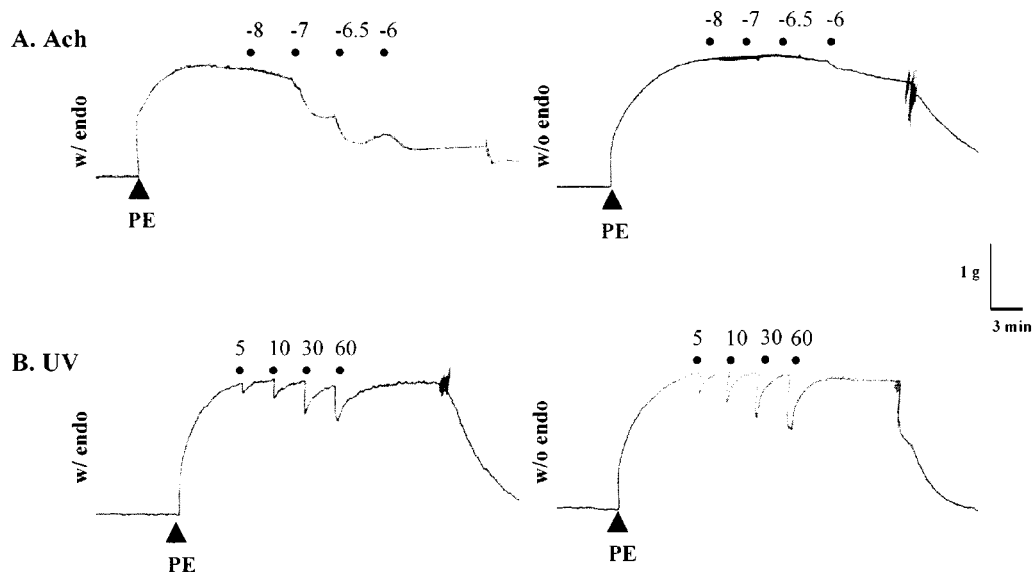


Fig. 1. Effects of UV light irradiation on vascular relaxation of rat aorta. Phenylephrine (PE)-induced precontraction in a rat aorta, which had been incubated in normal Krebs solution with endothelium (w/ endo) and denuded endothelium (w/o endo). A. The tracing of acetylcholine (Ach, numbers indicate log molar concentration)-induced relaxation in w/endo and w/o endo preparations. B. The tracing of UV light-induced photorelaxation in which the numbers indicate UV light exposure time (seconds) in w/ endo and w/o endo preparations.

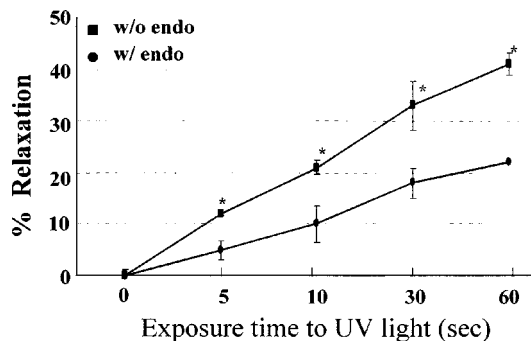


Fig. 2. Potentiation of photorelaxation in endothelium denuded-rat aorta. The aortic rings were relaxed in an exposure-time dependent manner. The results are measured as peak amplitudes and expressed as percentages of the phenylephrine-induced contraction in the same strips. Values represent the means of 3 separated experiments performed. w/endo represents the endothelium present and w/o endo represents the endothelium denuded rat aortas.

Effect of Ca^{2+} -free and verapamil containing medium on UV light-induced photorelaxation

As it has been reported that nitric oxide (NO) is a second messenger molecule with diverse functions, such as, vasodilatation [20], neurotransmission [21] and platelet aggregation [22]. It is formed in an oxygen-dependent reaction during which L-arginine is converted into L-citrulline by the enzyme, NO synthase (NOS). The three major categories of the enzyme regulating NO production are the constitutive, calcium-dependent isoforms principally present in endothelial and neuronal cells (eNOS and

nNOS, respectively), and the inducible, calcium-independent isoform (iNOS) first described in murine macrophage [23].

Thus, to understand which isoform acts as messenger in UV light-induced photorelaxation, Ca^{2+} -free and Ca^{2+} -channel blocker, verapamil were introduced in rat aortas. The magnitude of the potentiation of UV light-induced photorelaxation was significantly decreased in the Ca^{2+} -free medium, and completely diminished in verapamil treated aorta (Figure 5).

Expression and localization of eNOS and iNOS in UV light irradiated rat aortas

eNOS and iNOS expression were determined by the immunodetection of the anti-eNOS and iNOS antibodies, which are immunohistochemically specific monoclonal antibodies. eNOS and iNOS expression were increased upon UV light exposure in a time-dependent manner (Figure 6A, 6B). eNOS immunoreactivity was exclusively detected in endothelium, whereas iNOS was detected in both endothelium and aortic smooth muscle.

Discussion

The impairment of endothelial function is associated with the decreased production of NO and/or a concomitant release of endothelial contracting factors which impair the affect of NO. The endothelial dysfunction observed in hypertension appears to be a consequence of high blood pressure since a variety of antihypertensive treatments

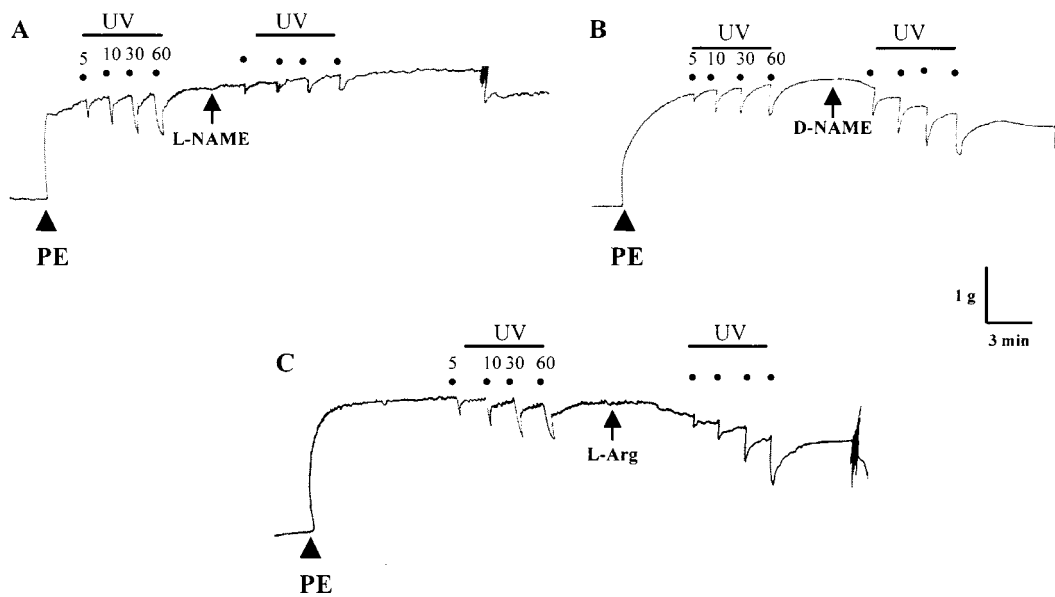


Fig. 3. Effects of L-NAME (A. 100 μ M), D-NAME (B. 100 μ M) and L-Arg (C, 10 μ M) on the UV light-induced photorelaxation of rat aortas.

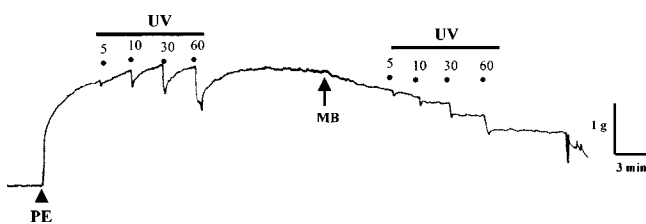


Fig. 4. Inhibitory effect of methylene blue (MB, 10 μ M) on UV light-induced photorelaxation of rat aorta.

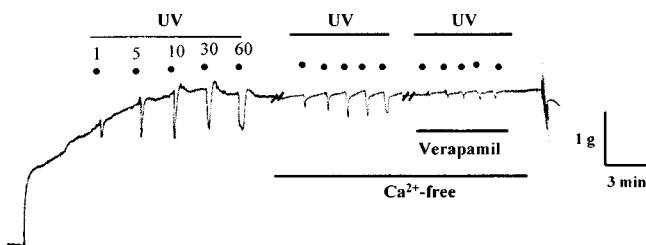


Fig. 5. Tracing of the inhibitory action of Ca^{2+} -free and verapamil (0.1 μ M) on UV light-induced photorelaxation.

normalize these responses. However, endothelial dysfunction may amplify the increase in vascular resistance since the inhibition of NO release causes an increase in blood pressure. The present study implicated that UV light-induced photorelaxation is may be due to endothelium-dependent and independent relaxants (Figure 1). We have shown, however, that acetylcholin (ACh)-induced vascular relaxation is absolutely endothelium dependent, but not in UV light-induced photorelaxation (Figure 1). The magnitude of the potentiation of photorelaxation is rather greater in endothelium-denuded

than endothelium-intact rat aortas (Figure 1, 2). Although, this did not unequivocally indicate that other factors in addition to endothelium derived relaxing factor (EDRF) exist, it at least showed that smooth muscle is related to UV light-induced photorelaxation.

Acetylcholine (ACh) produces relaxation in blood vessels via an endothelium-dependent mechanism [1]. Nitric oxide (NO) is an important factor involved in this response and is released from the endothelium following the binding of ACh to muscarinic receptors [24]. NO diffuses to the adjacent smooth muscle cells where it stimulates soluble guanylyl cyclase activity leading to increased cGMP levels [14]. To demonstrate the characteristic of UV light-induced photorelaxation, the administration of NOS inhibitor, L-NAME, its derivative, D-NAME, and NO donor, L-arginine, significantly induced the magnitude of potentiation of photorelaxation on L-NAME treatment, but it was not decreased with D-NAME treatment, in contract with the L-NAME treatment, photorelaxation was slightly increased by D-NAME treatment (Figure 3). Also, treatment with L-arginine significantly augmented the magnitude of the potentiation of photorelaxation. This result is consistent with previous reports [4, 6, 7].

The present study shows the significant inhibitory effect of methylene blue upon UV light-induced photorelaxation (Figure 4). These findings imply that UV light-induced photorelaxation is due to an interaction of the NOS and cGMP pathways. These result coincide with the suggestion of Furchgott *et al* [25] that UV light irradiation of vascular smooth muscle (photorelaxation) produces a labile photo-induced relaxing factor (PIRF) which, similar to

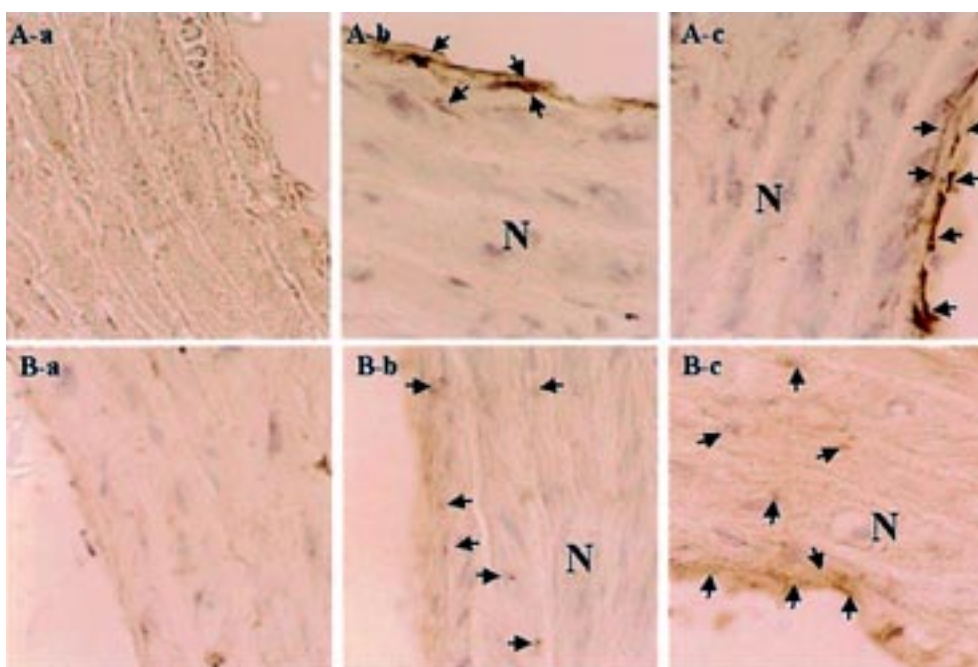


Fig. 6. Immunohistochemical expression and localization of eNOS and iNOS in UV light-irradiated rat aortas. eNOS and iNOS expression is shown in A and B panels, respectively. a: control, b: UV light irradiation for 30 sec. c: UV light irradiation for 3 min. Arrows indicate eNOS and iNOS localization, N indicates nucleus (5400). See "Materials and Methods" for further details.

endothelium-derived relaxing factor (EDRF), elevates cGMP levels and induces relaxation. It has been reported that vascular smooth muscle contains a depletable store of NO which is light-activated and restored by NO donors [26].

NO is formed in an oxygen-dependent reaction during which L-arginine is converted into L-citrulline by the enzyme, NO synthase (NOS). The three major categories of the enzyme regulating NO production are the constitutive, calcium-dependent isoforms principally present in endothelial and neuronal cells (eNOS and nNOS, respectively), and the inducible, calcium-independent isoform (iNOS) first described in murine macrophage [23].

Thus, to determine which isoform acts as messenger in UV light-induced photorelaxation, Ca^{2+} -free and Ca^{2+} -channel blocker, verapamil were introduced in rat aortas. The magnitude of potentiation of UV light-induced photorelaxation was significantly decreased in Ca^{2+} -free medium, and completely diminished in verapamil treated aorta (Figure 5). This result means that the majority of the UV light-induced photorelaxation might be due to Ca^{2+} -dependent relaxing factor. It suggests the plausibility of a relation involving Ca^{2+} -dependent NOS such as eNOS and nNOS, except inducible NOS (iNOS).

Thus, we examined the expression and localization of iNOS and eNOS using monoclonal antibodies on UV light irradiation in rat aortas (Figure 6). As shown in Figure 6, we detected the expression of eNOS in the endothelium,

whereas, the expression and localization of iNOS was in the endothelium and smooth muscle. Finally, UV light-induced photorelaxation is due to the expression and activation of Ca^{2+} -dependent NOS isoforms such as eNOS and nNOS but not iNOS.

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