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Involvement of nitrosothiols, nitric oxide and voltage-gated K^+ channels in photorelaxation of vascular smooth muscle

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Abstract

The effects of nitrosothiol depleting compounds (*p*-hydroxymercuribenzoate, iodacetamide and ethacrynic acid), a guanylyl cyclase inhibitor (1*H*[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one, ODQ) and nitric oxide (NO) scavenger agents (xanthine/xanthine oxidase and 2-(4-carboxyphenyl)-4,4,5,5-tetramethyl-imidazoline-1-oxyl-3-oxide; carboxy-PTIO) on light-induced photorelaxation in rat thoracic aorta were investigated. Photorelaxation responses were decreased in the presence of nitrosothiol depleting compounds suggesting *S*-nitrosothiols as the tissue source of the NO, whereas reduction in photorelaxation by the guanylyl cyclase inhibitor and NO scavenger agents indicates involvement of both NO and cGMP in photorelaxation. In addition the sensitivity of photorelaxation to the voltage-gated potassium channel (K_V) inhibitor, 4-aminopyridine, indicates that photorelaxation is mediated via a NO/cGMP-dependent, and, perhaps, direct light, activation of K_V channels. © 1998 Elsevier Science B.V.

Keywords: Photorelaxation; Nitrosothiol; Nitric oxide (NO); Guanylyl cyclase; K^+ channel

1. Introduction

In 1955 Furchgott reported that rabbit aorta precontracted with a vasoactive agent such as norepinephrine relaxed reversibly during exposure to strong illumination (Furchgott et al., 1955). Subsequently it was reported that non-vascular smooth muscle is insensitive, or much less sensitive to light, for instance, rabbit, cat and dog stomach and uterine smooth muscle do not show photorelaxation (Ehrreich and Furchgott, 1968).

Photorelaxation is accompanied by an increase in cyclic GMP and methylene blue inhibits this endothelium-independent relaxation and the associated UV-light dependent increase in cGMP production (Furchgott et al., 1985). Inhibition of photorelaxation slowly develops after addition of haemoglobin (Furchgott et al., 1985) and 6-(phenylamino)-5,8-quinolinedione, LY 83583 (Furchgott and Jothianandan, 1991). Agents which depolarize the vascular smooth muscle cell, such as ouabain, tetraethylammonium or KCl inhibit photorelaxation (Charpie et al., 1994), whereas 3-nitro-1,4-dihydropyridines and *N*-nitroarginine, that can be decomposed by light exposure to release NO, enhance photorelaxation (Chen and Gillis, 1992; Lovren et al., 1996).

Since the NO synthase inhibitor *N*-monomethyl-L-arginine acetate, L-NMMA does not inhibit photorelaxation and superoxide dismutase had no effect on photorelaxation (Matsunaga and Furchgott, 1989), it has been suggested that photorelaxation is independent of the L-arginine/NO pathway (Chang et al., 1993). Recently, Goud et al. (1996) provided additional data to refute the hypotheses that photorelaxation is mediated by NO and/or cGMP production, vascular smooth muscle cell hyperpolarization, or by an alteration in drug-receptor binding, thus suggesting that a novel vasodilator mechanism is responsible for photorelaxation. However, NO release from vascular smooth muscle in response to exposure to UV light has been confirmed by measurement with a porphyrinic sensor (Kubaszewski et al., 1994). Kubaszewski et al. speculated that a photoactivable NO store, perhaps NO bound to thiols, non-heme iron or nitrite was released by light. Venturini et al. (1993) also suggested that the photorelax-

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ation is mediated, in part, by a pool of light-activated vasodilatator that may be a store of NO which could play a role in the regulation of vascular tone. Megson et al. (1995) reported that photorelaxation is caused by the release of NO from a photosensitive nitrosothiol(s) store that can be exhausted rapidly by irradiating with laser light.

S-nitrosothiols are compounds that under appropriate conditions decompose to liberate nitric oxide and the corresponding disulfide (Ignarro et al., 1981). It has been suggested that the formation and decay of low molecular weight *S*-nitrosothiols, such as *S*-nitrosoglutathione and *S*-nitrosocysteine may represent a mechanism for the storage or transport of NO (Myers et al., 1990; Girard and Potier, 1993). Decomposition of the *S*-nitrosothiols and subsequent NO release is thus followed by the corresponding biological effect (Josephy et al., 1984). It has been established that *S*-nitrosothiols are sensitive to both photolytic (Singh et al., 1995) and breakdown catalysed by transition metal-ions (McAninly et al., 1993).

Assuming the existence of NO stores in vascular tissue, we investigated the possible contribution of *S*-nitrosothiols as NO stores in photorelaxation. *S*-nitrosothiols are unstable in an alkaline medium and, furthermore, can be decomposed by *p*-hydroxymercuribenzoate, iodacetamide and sodium iodide. In the present study an alkaline pH or presence of iodoacetamide or *p*-hydroxymercuribenzoate resulted in an incomplete inhibition of photorelaxation. However, ethacrynic acid, a thiol alkylating agent (Needleman et al., 1973), completely abolished the photorelaxation suggesting a possible role for free thiols in photorelaxation. Our observations with experimental protocols that lead to the inactivation of *S*-nitrosothiols also decreased photorelaxation, suggest that *S*-nitrosothiols do contribute to the photorelaxation process.

In the present study, the possibility that NO mediates photorelaxation was examined in the rat thoracic aorta with agents which have previously been shown to inhibit NO-mediated relaxation, such as xanthine/xanthine oxidase, 2-(4-carboxyphenyl)-4,4,5,5-tetramethyl-imidazoline-1-oxyl-3-oxide (carboxy-PTIO), and the selective guanylyl cyclase inhibitor 1*H*[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ). Our results with NO scavenger agents and guanylyl cyclase inhibitor indicate the role for both NO and guanylyl cyclase in photorelaxation. In some of these experiments 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-5-pyridinecarboxylic acid methyl ester, BAY K 8644 was also used as a light-sensitive source of NO (Lovren et al., 1996).

To further investigate the mechanism of photorelaxation, we tested whether photorelaxation is dependent on vascular smooth muscle hyperpolarization and potassium channel activity. The dependence of photorelaxation on potassium concentrations suggests that K^+-efflux through K^+-channels is involved in this effect. Furthermore, 4-aminopyridine (0.5 mM) which blocks voltage-gated K^+ channel (K_V), significantly inhibited photorelaxation. The ATP-sensitive K^+-channel blocker glibenclamide (10 μM) and Ca^{2+}-activated K-channel blocker tetraethylammonium (1 mM), however, had no effect on photorelaxation. These data suggest that, photorelaxation is due, primarily to cellular hyperpolarization and activation of K_V channels.

2. Materials and methods

2.1. Tissue preparation

Thoracic aortae were obtained from Sprague–Dawley rats (Charls River, PQ, Canada; 250–350 g) after animals had been stunned by a blow to the head and exsanguinated in accordance with guidelines established by the University of Calgary Animal Care Committee. The preparations were cleaned of all connective tissues and cut into rings approximately 3–4 mm length. Each ring was mounted under 2 g passive tension in 25 ml organ baths containing physiological salt solution (PSS) maintained at 37°C and bubbled with 95% O_2/5% CO_2. Isometric tension was recorded with a force displacement transducer (Grass FT .03) coupled to a Grass polygraph model 7D. The PSS used had the following composition (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 12.5 and glucose 11.1. Tissues were routinely allowed to equilibrate for 1 h before start of the experiments. The following data were obtained from experiments with endothelium-intact preparations.

2.2. Light source

The radiation source for photorelaxation was a halogen dissecting lamp generating polychromatic light (Nikon MK 50). Light was beamed through a bifurcated fibre optic light guide at the tissues for 30 s every 3–4 min. The distance from the light guide to an aortic preparation was 4–5 cm. Light intensity from the polychromatic light source was 0.17 W cm^{-2} at the tissue. Light intensity was measured with a radiometer (IL 1700, Ealing Scientific).

The fluorescent lighting in the laboratory was turned off, after tissue dissection and preparation and during all experiments.

2.3. Experimental protocols

2.3.1. Irradiation of vascular tissue

Experiments in this series were conducted in order to examine the influence of light stimulation on active tone either in the presence or absence of the dihydropyridine Bay K 8644. For this purpose, aortic ring preparations were precontracted with 10 nM phenylephrine that produced a half maximal response (EC_{50}), and the photorelaxation response measured. In the experiments with Bay K

8644 when tone was increased, 0.1 μM Bay K 8644 was added and photorelaxation response measured. It should be noted that once the tissue preparation has been treated with Bay K 8644, the Bay K 8644-induced photorelaxation is longlasting and cannot be eliminated by repeated washout with drug-free PSS. In each experiment, one aortic ring tissue served as a control and was only exposed to phenylephrine or phenylephrine and Bay K 8644.

2.3.2. Effects of p-hydroxymercuribenzoate, iodacetamide and sodium iodide on photorelaxation

The effects of nitrosothiol-depleting compounds such as *p*-hydroxymercuribenzoate (10 μM), sodium iodide (1–50 μM), iodacetamide (10 μM) and ethacrynic acid (50 μM) on light-induced photorelaxation were tested. The aortic rings were incubated with individual *S*-nitrosothiol depleting compounds for 30 min, tone was raised with phenylephrine and photorelaxation responses determined.

2.3.3. Effect of elevated pH on photorelaxation

To examine the effect of alkaline medium on the photorelaxation, the aortic ring preparations were exposed for 1 h to modified PSS, pH 9.0–9.4 (in which the bicarbonate was replaced by Tris base adjusted to pH with HCl and bubbled with 100% oxygen (Needleman et al., 1973), then after the washing the tissues every 15 min with PSS for 1 h, photorelaxation was determined.

2.3.4. Effects of xanthine / xanthine oxidase on photorelaxation

The effect of superoxide anions (NO scavengers) on the photorelaxation responses were examined. Superoxide anions were generated by incubation of xanthine (2 mM) plus xanthine oxidase (10 mU ml^{-1}) in PSS for 1 h. Xanthine/xanthine oxidase in PSS was added to aortic tissues, vascular tone was then raised by the addition of phenylephrine. The effects of superoxide anions on intrinsic and Bay K 8644 (0.1 μM)-induced photorelaxation in precontracted tissue were performed.

2.3.5. Effects of carboxy-PTIO and ODQ on photorelaxation

This series of experiments was designed with the objective to determine whether irradiation induces release of NO which then activates soluble guanylyl cyclase. The effects of a 30 min preincubation of the tissues with the NO scavenger carboxy-PTIO (50 μM) and the soluble guanylyl cyclase inhibitor ODQ (10 μM) were assessed in phenylephrine (10 nM) precontracted tissues.

2.3.6. Effects of extracellular K^+ concentrations and K^+-channel blockers on photorelaxation

Experiments in this series examined the influence of varying extracellular K^+ concentrations on photorelaxation. To compare photorelaxation in all preparations tone was increased to the same level (half maximal response) either with phenylephrine or high potassium PSS and phenylephrine. To determine which K^+ channels might be involved in the K^+ efflux associated with photorelaxation, the following K^+ channel blockers were incubated with tissues for 30 min: 4-aminopyridine (0.5 mM), tetraethylammonium (1 mM) and glibenclamide (0.01 mM).

2.4. Drugs

The following compounds were used: Bay K 8644 ((±)1.4-dihydro-2.6-dimethyl-3-nitro-4-(2-trifluoromethyl)-5-pyridinecarboxylic acid methyl ester) and carboxy-PTIO (2-(4-carboxyphenyl)-4,4,5,5-tetramethyl-imidazoline-1-oxyl-3-oxide potassium), RBI, Natick, MA, USA; ODQ (1*H*[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one) Torcis, Ballwin, MO, USA, and all other compounds were from Sigma, St. Louis, MO, USA.

2.5. Data analysis

Data have been expressed as mean ± S.E.M. of the percentage maximal responses for *n* experiments. The effects of various interventions were studied by comparing an untreated control with a treated tissue and differences

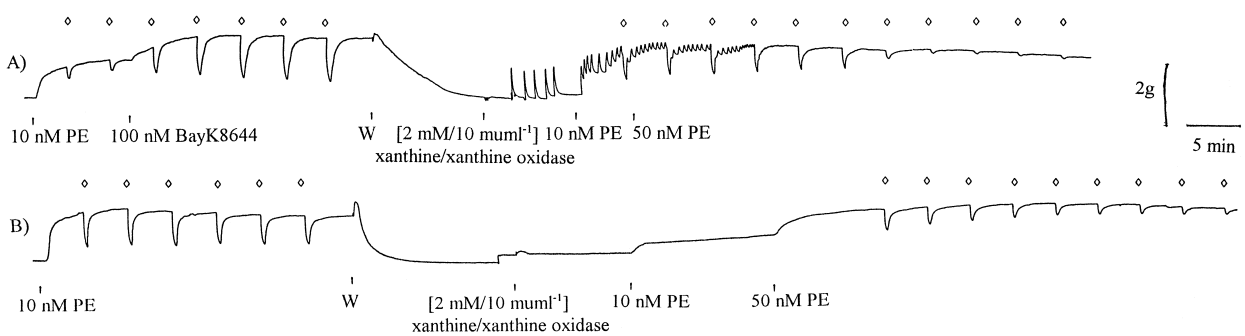

Fig. 1. Effects of xanthine/xanthine oxidase on photorelaxation in the rat aortic rings in the presence of (A) and absence of Bay K 8644 (B). (◇) Indicates light stimulation.

Table 1
Effects of NO scavengers and guanylyl cyclase inhibitor on photorelaxation[a]

Photorelaxation	Control	ODQ (10 μM)	Carboxy-PTIO (50 μM)	X/XO (2 mM/10 mU ml^{-1})
Light-induced	37.7 ± 7.4 (n = 15)	3.7 ± 6.0 (n = 5)	21.5 ± 5.3 (n = 4)	4.9 ± 1.7 (n = 5)
Bay K 8644-induced	72.7 ± 4.5 (n = 12)	46.7 ± 7.0 (n = 5)	ND	8.5 ± 5.3 (n = 8)

[a] Photorelaxation is presented as % inhibition of phenylephrine-induced active tone.
ND not determined.

between the mean values were determined by Student's *t*-test for paired observations, and were regarded as significant when $P < 0.05$.

3. Results

3.1. Irradiation of vascular tissue

Aortic rings precontracted with 10 nM phenylephrine were relaxed in a reversible and reproducible manner by light irradiation with maximal relaxation responses of 38 ± 7% (expressed as percentage relaxation of the maximal contractile response to phenylephrine). When Bay K 8644 was added to the tissue preparations, photorelaxation was enhanced to 73 ± 5% (Fig. 1).

3.2. Effects of p-hydroxymercuribenzoate, iodacetamide and sodium iodide on photorelaxation

10 μM *p*-hydroxymercuribenzoate significantly ($P < 0.05$) reduced photorelaxation from 38 ± 7 to 23 ± 2%. *p*-hydroxymercuribenzoate also depressed the phenylephrine induced tone by 15 ± 5%. Pretreatment of the tissue preparations with 10 μM iodacetamide resulted in reduction of the vascular contractility to phenylephrine by 18 ± 8%, and significantly ($P < 0.05$) decreased photorelaxation to 23 ± 6%. Incubation with sodium iodide (1–30 μM) did not affect photorelaxation responses and *p*-hydroxymercuribenzoate and iodacetamide in concentrations lower than 10 μM did not significantly affect photorelaxation. In concentrations higher than 10 μM *p*-hydroxymercuribenzoate and iodacetamide irreversibly blocked phenylephrine mediated contractions and hence their effects on photorelaxation could not be demonstrated.

3.3. Effects of ethacrynic acid on photorelaxation

After pre-incubation with ethacrynic acid (50 μM) no changes in the tissue contractility were observed, but photorelaxation responses were reduced by 93 ± 2%.

3.4. Effect of elevated pH on photorelaxation

Photorelaxation responses in the tissue preparations treated with modified PSS, pH 9.0–9.4 and bubbled with 100% oxygen were significantly reduced, 21 ± 8% vs. 39 ± 7% in control ($P < 0.05$). In 30% of the ring preparations tested this treatment irreversibly blocked tissue contractions and in all others the contractile responses to phenylephrine were decreased by 33 ± 8%.

3.5. Effects of xanthine / xanthine oxidase on photorelaxation

The attenuation of the intrinsic or Bay K 8644 induced photorelaxation in the presence of xanthine/xanthine oxidase (2 mM/10 mU ml^{-1}) developed slowly with the magnitude significantly reduced by 95 ± 2% and 92 ± 5%, respectively.

3.6. Effects of carboxy-PTIO and ODQ on photorelaxation

50 μM carboxy-PTIO reduced intrinsic photorelaxation in precontracted rat aortic ring preparations. Light-induced relaxation was almost completely abolished after treatment with ODQ, whereas the magnitude of the photorelaxation induced by Bay K 8644 was significantly reduced, but to a significantly lower extent than in the absence of Bay K 8644, $P < 0.0001$ and < 0.05, respectively. (Table 1).

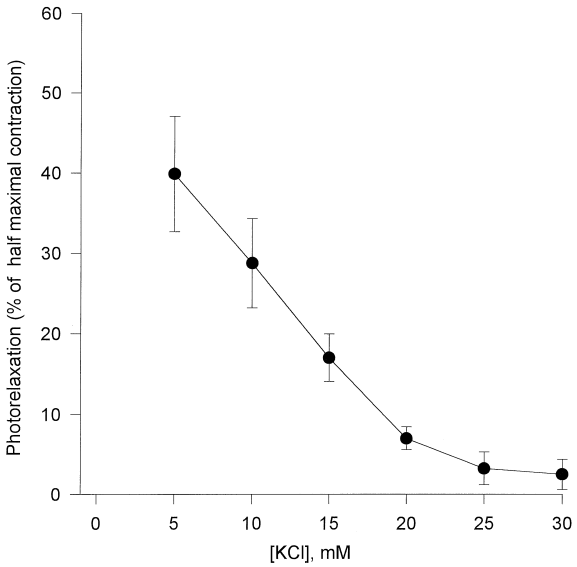

Fig. 2. Effect of extracellular potassium (final bath) concentration on photorelaxation in the precontracted rat aortic preparations. Data represent mean ± S.E.M. (n = 5).

3.7. Effects of extracellular K^+ concentrations and K^+-channel blockers on photorelaxation

In preparations where tone was increased to the half maximal level with phenylephrine (10 nM) or elevated K^+ concentrations (5–25 mM) with phenylephrine (0.5–5 nM), photorelaxation was reduced only in the presence of increasing concentrations of extracellular KCl (Fig. 2).

4-aminopyridine (0.5 mM), a relatively selective blocker of K_V channels, significantly inhibited photorelaxation without affecting the basal tension, 21 ± 4 vs. $40 \pm 8\%$ in control ($P < 0.005$). The K_{ATP} channel blocker glibenclamide (0.01 mM), and Ca^{2+}-activated K^+-channel blocker tetraethylammonium (1 mM), however, did not significantly inhibit photorelaxation, 32 ± 8 and 36 ± 9 vs. $40 \pm 8\%$ in control, respectively ($P > 0.05$).

4. Discussion

S-nitrosothiols have many biological activities and have been suggested to be intermediates in signal transduction (Upchurch et al., 1995) and photorelaxation (Kubaszewski et al., 1994). In these studies we evaluated the possible contribution of *S*-nitrosothiols to photorelaxation.

The stability of *S*-nitrosothiols in solutions varies markedly depending on temperature, pH and the presence of oxygen. Maximal stability has been observed at 0–4°C in the absence of oxygen and physiological pH, whereas minimal stability occurred at 37°C in the presence of oxygen and increased pH (Ignarro et al., 1981). In our experiments the exposure of the ring preparations to modified PSS, at a pH 9.0–9.4 bubbled with pure oxygen, decreased the photorelaxation by $43 \pm 7\%$ of control thus suggesting that *S*-nitrosothiols are partially involved in the photorelaxation. However, because *S*-nitrosothiols should be extremely unstable in the alkaline pH in our experimental conditions, a higher inhibition of photorelaxation should have been expected if photorelaxation exclusively resulted from *S*-nitrosothiol decomposition.

S-nitrosothiols are also sensitive to photolytic and transition metal ion-dependent breakdown. It is generally assumed that they decompose by homolytic cleavage of the S–N bond resulting in the release of NO and the thiyl radical (Singh et al., 1996). UV–visible light causes homolytic cleavage of the sulfur–nitrogen bond, resulting in the release of nitric oxide and the thiyl radical. Transition metal ions also induce decomposition of *S*-nitrosothiols.

Mercury ions selectively displace NO from *S*-nitrosothiols producing very strong complexes (Saville, 1958). It has been shown in bioassay cascade studies that $HgCl_2$, without affecting vascular tone, abolished *S*-nitrosothiol induced relaxations in the rabbit aorta (Hecker et al., 1995). Since aromatic organic mercurials have the same effects as $HgCl_2$ (Jocelyn, 1972), we used *p*-hydroxymercuribenzoate to avoid the significant depression of tissue contractility which we observed with $HgCl_2$ (data not shown). Although in our experiments *p*-hydroxymercuri benzoate did depress tissue contractility, the greater inhibition of photorelaxation provides additional evidence for a role for *S*-nitrosothiols in photorelaxation.

Cellular thiols are alkylated under physiological conditions by various alkyl halogens (Jocelyn, 1972). Additionally, it has been shown that reflux with sodium iodide cleaved nitric oxide from *S*-nitrosocysteine (Myers et al., 1990). Our studies indicated that photorelaxation was not changed when the tissues were pretreated with 10 μM sodium iodide, but that 10 μM iodacetamide significantly reduced photorelaxation again suggesting the involvement of *S*-nitrosothiols in photorelaxation. The failure of sodium iodide to reduce photorelaxation in our experimental protocols may reflect our inability to reproduce the reflux conditions required for the chemical interaction to occur in the organ bath.

Experiments with the thiol alkylating agent ethacrynic acid have illustrated the importance of tissue free thiol groups in organic nitrate-induced vasodilatation (Needleman et al., 1973). In our study following exposure to ethacrynic acid, the photorelaxation was almost completely abolished suggesting the significance of the free thiol groups in the photorelaxation. The almost complete inhibition of photorelaxation with ethacrynic acid may reflect its reaction with both, free thiols and *S*-nitrosothiols.

The results from this study provide additional evidence that light induced relaxation in precontracted rat aorta results from the generation of NO and subsequent activation of soluble guanylyl cyclase and, in addition, that *S*-nitrosothiols are likely the tissue source of the NO. Our data also suggests that photorelaxation depends, at least in part, on activation of K_V channels.

Carboxy-PTIO reacts with biologically derived nitric oxide to form carboxy-PTI derivatives (Akaike et al., 1993). The effect of this scavenger of free radical nitric oxide was to reduce intrinsic photorelaxation by $43 \pm 6\%$, thus supporting the hypothesis that NO is a mediator of photorelaxation. Carboxy-PTIO rapidly inactivates NO by forming the corresponding imidazolineoxyl and NO_2 free radical, which in the turn reacts with water to form hydrogen ions, nitrite and nitrate ions (Akaike et al., 1993). Nitrite and nitrate ions, however, can release nitric oxide in the presence of the light, thus inducing photorelaxation, and in consequence, the scavenger effect of carboxy-PTIO was lower when compared with the scavenger effect of xanthine/xanthine oxidase.

Xanthine/xanthine oxide is a generator of superoxide anion that very effectively scavenges NO, generating peroxynitrite (Blough and Zafirou, 1985). In an earlier study we reported that xanthine/xanthine oxide significantly decreased, but did not completely abolish Bay K 8644-induced photorelaxation, suggesting that the NO generation is involved in Bay K 8644-dependent photorelaxation (Lovren et al., 1996). In the present study

xanthine/xanthine oxide attenuated relaxations induced by light exposure from 38 ± 7 to $4 \pm 1\%$, thus providing additional evidence that NO mediates photorelaxation.

ODQ inhibits NO-induced increases in cyclic GMP concentrations in the brain (Garthwaite et al., 1995). Furthermore, ODQ selectively inhibits soluble guanylyl cyclase in vascular tissue and platelets without a direct effect on NO (Moro et al., 1966; Olson et al., 1997) and thus is more selective than other soluble guanylyl cyclase inhibitors such as methylene blue or LY 83583. In our studies ODQ almost completely inhibited photorelaxation and reduced Bay K 8644-induced photorelaxation, $90 \pm 6\%$ and $36 \pm 5\%$, respectively, thus indicating the involvement of soluble guanylyl cyclase activation in light-induced relaxations. Although methylene blue and LY 83583 also reduce photorelaxation response, the effect of ODQ was much more rapid and complete. ODQ is more potent inhibitor of intrinsic photorelaxation then Bay K 8644-induced photorelaxation, suggesting that Bay K 8644-induced photorelaxation involves an additional non-NO mediated mechanism.

Previously, it was demonstrated that photorelaxation was significantly reduced when contraction had been produced by the addition of KCl (15 mM) (Furchgott et al., 1961). Charpie et al. (1994) observed that incubation with KCl (40 mM), ouabain (1 mM) and tetraethylammonium (20 mM) inhibited photorelaxation and speculated that the release of a photo-induced relaxing factor from smooth muscle, similar to endothelium-derived hyperpolarizing factor, is responsible for photorelaxation. Recently, Chang et al. (1997) have shown that photo-induced relaxing factor and membrane hyperpolarization are involved in photorelaxation. However, contradicting the hypothesis that photorelaxation is mediated by vascular smooth muscle cell hyperpolarization Goud et al. (1996) presented data that indicated that there is no significant difference in the level of photorelaxation in phenylephrine vs. KCl contracted tissues. In our experiments photorelaxation was reduced by increasing extracellular KCl concentrations thus suggesting dependence of photorelaxation on smooth muscle hyperpolarization (Chen and Suzuki, 1989). Moreover, the dose-dependent inhibition of intrinsic photorelaxation by 4-aminopyridine, which is the most selective known inhibitor of K_V channels (Beech and Bolton, 1989), suggests that photorelaxation is mediated by the activation of K_V channels. Tetraethylammonium, a selective blocker of K_{Ca} channels, inhibits K_V channels at higher concentrations than it inhibits K_{Ca} channels (> 5 mM) (Robertson and Nelson, 1994). Glibenclamide, an inhibitor of ATP-sensitive K^+ channels, inhibits K_V channels in rabbit portal vein but only at a thousandfold greater concentration than required to inhibit K_{ATP} channels (Beech et al., 1993). Since in our experiments photorelaxation was unaffected by either glibenclamide (10 μM) or tetraethylammonium (1 mM), it is unlikely that K_{ATP} or K_{Ca} channels are involved in the process. Furthermore, since photorelaxation (non-dihydropyridine-enhanced) is almost completely inhibited by the guanylyl cyclase inhibitor ODQ and the K_V channel inhibitor 4-aminopyridine, we conclude that light-induced opening of the K_V channel is mediated by a cGMP-dependent process.

This study has demonstrated that photorelaxation is mediated, at least in part, by NO released by the light-induced decomposition of *S*-nitrosothiols. However, the incomplete inhibition of photorelaxation by a variety of *S*-nitrosothiol depleting compounds also suggests that an additional source of NO may be released by light and contribute to photorelaxation. Furthermore, the present study provides evidence that photorelaxation is dependent on the activation of 4-aminopyridine-sensitive K_V channels. In conclusion, the present study has demonstrated that NO, released from *S*-nitrosothiols, mediates photorelaxation in the rat aorta via a, primarily, guanylyl cyclase dependent opening of K_V channels. However, a contribution to photorelaxation by a direct NO and/or light-activation of K channels cannot be entirely ruled out.

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