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Nitric oxide, a possible mediator of 1,4-dihydropyridine-induced photorelaxation of vascular smooth muscle

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1 In rat aortic tissues pre-contracted with phenylephrine, certain 1,4-dihydropyridines (DHPs) such as Bay K 8644 (0.1 μM), PN 202791 (1 μM), RK 30 (1 μM), NI 104 (1 μM) and NI 105 (1 μM) enhanced photoactivated relaxations (photorelaxation or PR) whereas NI 72, NI 85, NI 99, NI 102, amlodipine, felodipine, nifedipine and nimodipine were inactive.

2 The PR inducing effects of Bay K 8644 were mimicked by the diabetogenic agent, streptozotocin (STZ).

3 Solutions of Bay K 8644 which had been irradiated for various periods of time initiated light independent transient relaxations followed by contractile responses in aortic tissue partially contracted with phenylephrine. With exposure times to light of 30 to 120 min, the intensity of the relaxation response to irradiated Bay K 8644 increased from 26 ± 3.3 to $71 \pm 3.7\%$ of the maximum contractile response to phenylephrine ($n=5$). Conversely the contractile responses decreased, from 84.2 ± 4.1 to $19.8 \pm 10.4\%$ of the maximum contractile response to phenylephrine ($n=5$).

4 Superoxide ions, generated by incubation of xanthine (2 mM) plus xanthine oxidase (10 mu ml^{-1}) in physiological saline solution (PSS) 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 (mM) for 1 h, reduced the PR induced by DHPs, STZ, and also NO-induced relaxations of rat aortic preparations.

5 Direct measurements of NO indicate that, following exposure to a polychromatic light source, equimolar concentrations (0.1 mM) of the DHP compounds that enhance PR, as well as STZ, photodegrade to release NO ($25 \pm 2 - 40.3 \pm 5.9$ nmol min^{-1}, $n=6$).

6 Structure-activity studies indicate that a nitro group at the -3 position of the dihydropyridine ring is essential for DHPs to support PR.

7 These data suggest that the photodegradation of DHPs and STZ leading to the release of NO provides the primary cellular process underlying the PR response.

Keywords: Vascular smooth muscle; photorelaxation; dihydropyridines (DHPs); nitric oxide; chemiluminescence

Introduction

The 1,4-dihydropyridine (DHP), nifedipine, is a very important cardiovascular drug that is used for the control of angina, hypertension and other vascular diseases (Triggle, 1992). Nifedipine can be viewed as a prototypical drug for several generations of DHP antagonists and activators, which are all potently active at the L-type calcium channel (Varadi *et al.*, 1995).

DHP L-type calcium channel activators and antagonists share many common structural and conformational features that allow them to bind to the same receptor associated with the α_1 subunit of the L-type channel; however, they may have varying affinities for different gating-states of the channel (Langs *et al.*, 1991). Of interest is that several investigators have shown that certain activators and antagonists of the L-type Ca^{2+} channel can also photosensitize smooth muscle to the relaxant effects of uv-light (Mikkelsen & Kazda, 1985; Golenhofen *et al.*, 1990; Triggle & Bieger, 1990). The mechanism by which DHPs induce PR has not been elucidated. It should also be noted that certain smooth muscles demonstrate an intrinsic sensitivity to light and will relax if exposed to an appropriate photostimulation (Furchgott *et al.*, 1961; 1985). It is not known whether the same cellular processes are responsible for intrinsic PR versus that induced by DHPs; however, it has been shown that the maximum spectral sensitivity of the intrinsic PR is 390 nm whereas the DHP photoactivated relaxation maximum is at 410 nm, thus suggesting differing mechanisms (Golenhofen *et al.*, 1990).

Mikkelsen & Kazda (1985) proposed that a photosensitive protein may be associated with the voltage-gated calcium channel and that light induces a switch in Bay K 8644, binding from an agonist to an antagonist site, thus producing PR. However, it has been demonstrated that both agonist and antagonist enantiomeric pairs of DHPs, Bay K 8644 and PN 202971 ((+)- and (−)-Bay K 8644, and (+)- and (−)-PN 202791, respectively), which possess widely different binding affinity for DHP receptors, are equally capable of enhancing PR and that PR is independent of the presence of the endothelium, and also does not involve a nitric oxide synthase pathway (Golenhofen *et al.*, 1990; Triggle *et al.*, 1991; Chang *et al.*, 1993).

Several compounds structurally unrelated to DHPs, including streptozotocin (STZ), sodium nitroprusside (SNP) and $NaNO_2$ can also sensitize smooth muscle to the relaxant effects of light (Matsunaga & Furchgott, 1989). This enhancement of light-induced relaxation is accompanied by an increase in intracellular guanosine 3′:5′-cyclic monophosphate (cyclic GMP) (Triggle *et al.*, 1991). Furthermore solutions of these compounds, as well as Bay K 8644, can be shown to degrade to yield nitric oxide (NO) following exposure to light (O'Neill *et al.*, 1993; Bauer & Fung, 1994).

It is generally accepted that NO is the endogenous activator of soluble guanylyl cyclase thereby generating cyclic GMP and

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inducing vasodilatation (Ignarro *et al.*, 1981; Marks *et al.*, 1991; Noack & Feelisch, 1991). It is thus tempting to conclude that certain DHPs can also act as donors of free NO (Golenhofen *et al.*, 1990), or an unstable NO intermediate (Triggle *et al.*, 1991), and act in a manner analogous to other nitrovasodilatators such as nitroglycerin. The observation of Bauer & Fung (1994), see above, would seem to support this hypothesis. However, the precise chemical processes involved in mediating PR have not been elucidated and there is a lack of correlation between the concentrations of Bay K 8644, or PN 202791, required to induce PR versus the approximately 100 × greater concentrations required to generate detectable levels of NO (Triggle *et al.*, 1991; Bauer & Fung, 1994).

In the present study we compared the effects of polychromatic light on the chemical transformation and biological activity of a number of DHPs and STZ.

Methods

Tissue preparation

The thoracic aortae were obtained from Sprague-Dawley rats (Charles River, PQ, Canada; 250–350 g) after the 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 tissues were cleaned of all connective materials 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. The pH of the solution after saturation with 95% O_2/5% CO_2 gas mixture was 7.4. Tissues were routinely allowed to equilibrate for 1 h before the start of the experiments. The results described in this manuscript were obtained with endothelium-intact preparations of arteries. The effects of DHPs and STZ were essentially the same in endothelium-denuded preparations (data not shown).

Irradiation of vascular tissue

The radiation source for PR 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 face of 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 lab lighting was turned off, after tissue dissection and preparation and during all experiments.

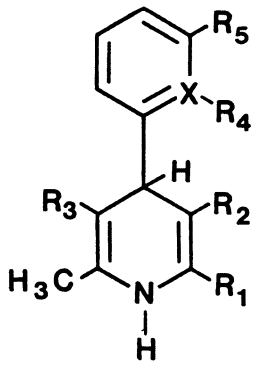
Experimental protocols

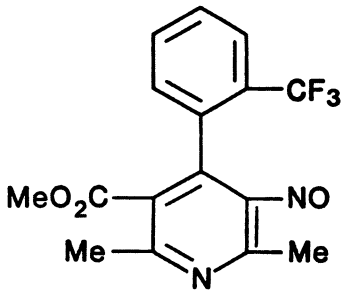
Protocol 1: Experiments were conducted in order to examine the influence of light stimulation on active tone in the presence of a number of DHPs including Bay K 8644, the enantiomeric pairs of PN 202791, as well as STZ. For this purpose, aortic ring preparations were precontracted with 10 nM phenylephrine to a moderate level of tone (EC_{50}), and the PR response measured.

Protocol 2: This series of experiments was designed to determine whether irradiation reduces/eliminates the L-type calcium channel activity of Bay K 8644 and concomitantly releases an NO-like product. Bay K 8644 solutions (1 mM) were placed in glass vials sealed with silicone septa and exposed to the light source at maximum power (0.9–1 W cm^{-2}) for periods of 30 min to 2 h. The effects of irradiated solutions of Bay K 8644, including the ability to support PR, were tested in phenylephrine (10 nM) precontracted tissues.

Protocol 3: The effects of superoxide anions (NO sca-

Table 1 Structural formulae of DHPs and their ability to support photorelaxation





N1 72

Compounds	R_1	R_2	R_3	R_4	R_5	X	*PR*
Bay K 8644	CH_3	NO_2	$COOCH_3$	CF_3	H	C	+
PN 202791	CH_3	NO_2	$COOCH(CH_3)_2$		=N-O-N=	C	+
RK 30	CH_3	NO_2	$COOCH(CH_3)_2$	–	H	N	+
NI 85	CH_3	$COO(CH_2)_2ONO_2$	$COOCH(CH_3)_2$	CF_3	H	C	–
NI 99	CH_3	$CONH_2$	$COOCH_3$	CF_3	H	C	–
NI 102	CH_3	$COO(CH_2)_2NHC(NH)NH_2$	$COOCH(CH_3)_2$	Cl	Cl	C	–
NI 104	CH_3	NO_2	$PO(OC_2H_5)_2$	CF_3	H	C	+
NI 105	CH_3	NO_2	$COCH_3$	CF_3	H	C	+
Amlodipine	$CH_2O(CH_2)_2NH_2$	$COOCH_2CH_3$	$COOCH_3$	Cl	H	C	–
Felodipine	CH_3	$COOCH_2CH_3$	$COOCH_3$	Cl	Cl	C	–
Nifedipine	CH_3	$COOCH_3$	$COOCH_3$	NO_2	H	C	–
Nimodipine	CH_3	$COOCH(CH_3)_2$	$COO(CH_2)_2OCH_3$	H	NO_2	C	–
NI 72							–

vengers) on the PR responses to DHPs, and STZ, and relaxation to Bay K 8644 solutions irradiated for 90 min 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 (X)/xanthine oxidase (XO) in PSS was added to aortic tissues pretreated with either a DHP or STZ, vascular tone was then raised by the addition of phenylephrine. The effect of superoxide anion on the NO (0.1 μM)-induced relaxation response in precontracted tissue was also assessed as a parallel control for the experiments with DHP and STZ.

Measurement of nitric oxide

Nitric oxide photolytically released from DHPs and STZ was quantitated by modification of the previously described chemiluminescence method (O'Neill *et al.*, 1993).

Solutions of Bay K 8644, nifedipine, nimodipine, amlodipine, felodipine and PN 202791 were placed in a purge trap vessel. The headspace of the vessel was attached to a Sievers model 270 nitric oxide chemiluminescence detector. Drug solutions (2.0 ml, 1×10^{-4} M) were continuously purged with argon gas through a glass frit at the bottom of the vessel, the argon gas stream stripped nitric oxide from solution into the headspace and then detector. Both fibre optic light guides from a Nikon MK 50 dissection lamp were positioned to irradiate the solution in the purge trap vessel. The lamp was set at maximum power (0.9–1.0 Wcm^{-2}).

The chemiluminescence detector was calibrated with a standard gas mixture of NO in nitrogen and a flowmeter (GF-4540, Gilmont Instruments Inc., Barrington, IL, U.S.A.) as follows: a standard mixture of nitric oxide (0.6582 nmol ml^{-1} NO) was run at various flow rates into the purge trap vessel. A standard curve of nmol NO min^{-1} vs. chemiluminescence detector response was then constructed in order to quantify the amount of nitric oxide photo-released from various drugs.

This method allows measurement of the rate of NO production in nmol min^{-1} as opposed to the measurement of NO from a bolus of the NO liberating compound.

Materials

The following agents were used: phenylephrine hydrochloride, streptozotocin, xanthine sodium salt, xanthine oxidase (Sigma Chemical Co., St. Louis, MO, U.S.A.) as aqueous solutions; (±)-Bay K 8644 (1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-5-pyridinecarboxylic acid methyl ester, RBI, Natick, MA, U.S.A.), (−)- and (+)-PN 202791 (1,4-dihydro-2,6-dimethyl-3-nitro-4-(2,1,3-benzoxadiazol-4-yl)-5-pyridinecarboxylic acid isopropyl ester, generous gift from Sandoz, Basel, Switzerland) as solutions in anhydrous ethyl alcohol (Commercial Alcohol Inc., Brampton, Ontario). Other DHPs, including amlodipine (Pfizer Central Research, Sandwich, England), felodipine (Hässle, Molndal, Sweden), nifedipine and nimodipine (RBI, Natick, MA, U.S.A.), and some DHPs and non-DHP, NI 72, synthesized by the Faculty of Pharm. Sci., Edmonton, AB, Canada (NI 72, 2,6-dimethyl-3-nitroso-4-(2-trifluoromethylphenyl)-5-pyridinecarboxylic acid methyl ester); NI 99 (1,4-dihydro-2,6-dimethyl-3-carboxamido-4-(2-trifluoromethylphenyl)-5-pyridinecarboxylic acid methyl ester); NI 102 (1,4-dihydro-2,6-dimethyl-3-(2-guanidinoethyl)-4-(2,3-dichloro-phenyl)-3,5-pyridinedicarboxylate 5-isopropyl ester); NI 104 (1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-5-pyridinephosphoric acid diethyl ester); NI 105 (1,4-dihydro-2,6-dimethyl-3-nitro-5-acetyl-4-(2-trifluoromethylphenyl)pyridinecarboxylic acid methyl ester); RK 30, (1,4-dihydro-2,6-dimethyl-3-nitro-4-(pyridine-2-yl)-5-pyridinecarboxylic acid isopropyl ester) were also prepared as solutions in anhydrous ethyl alcohol. Standard mixtures of 5–432 p.p.m. nitric oxide in nitrogen, ultrapure grade argon, nitrogen, medical grade oxygen and pure nitric oxide were obtained commercially (Linde Div. Union Carbide Calgary, AB, Canada).

Data analysis

Data have been expressed as mean ± s.e.mean of the percentage maximal responses for n experiments. Differences between the mean values were determined by Student's t test for paired and unpaired observations and were regarded as significant when $P < 0.05$.

Results

Effects of DHPs and STZ on photorelaxation

Aortic rings precontracted with 10 nM phenylephrine were relaxed in a reversible and reproducible manner by light irradiation (0.17 W cm^{-2}) with maximal relaxation responses of 25 ± 15% (expressed as percentage relaxation of the maximal contractile response to phenylephrine). To minimize intrinsic PR, prior to the photoactivation of DHPs or STZ, light in-

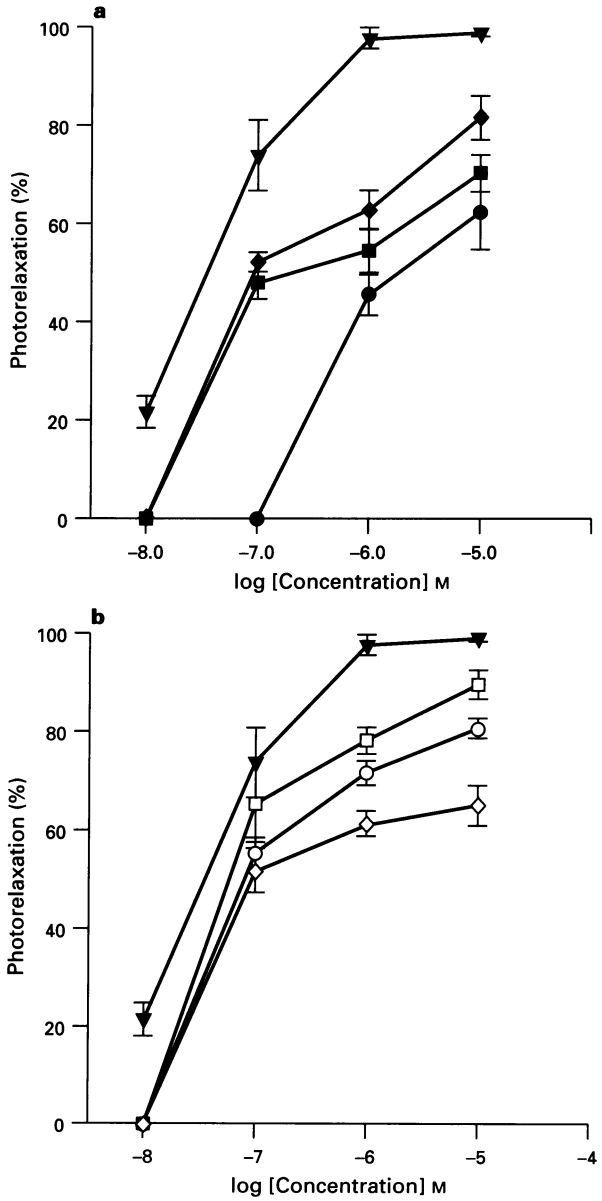


Figure 1 (a) Concentration-photorelaxation response curves for: (▼) Bay K 8644 vs. (◆) (−)-PN 202791; (■) (+)-PN 202791 and (●) STZ. Each point of the graph represents the mean ± s.e.mean ($n = 6$). (b) Concentration-photorelaxation response curves for (▼) Bay K 8644 vs. (□) RK 30; (○) NI 104; and (◇) NI 105. Each point of the graph represents the mean ± s.e.mean ($n = 6$).

tensity from the polychromatic light source was reduced. When DHP agonists such as (±)-Bay K 8644, (+)-PN 202791, RK 30, NI 104, or NI 105 were added to the tissue preparations, PR was enhanced whereas NI 85, NI 99, NI 102, and the DHP antagonists amlodipine, felodipine, nifedipine and nimodipine were inactive as well as the non-DHP compound NI 72 (Table 1). STZ directly relaxed phenylephrine-induced active tone; however, after restoring active tone to pre-STZ levels by increasing the concentration of phenylephrine (20 nM), the presence of STZ also resulted in PR. Both the calcium channel agonist and antagonist enantiomers of PN 202791 were more potent than STZ in inducing PR; furthermore the photorelaxant effect of DHPs could not be eliminated by repeated washout in drug-free PSS, whereas the effects of STZ were readily eliminated. Figure 1a and 1b compare and contrast the concentration-photorelaxation response curves of DHP compounds and STZ. DHPs and STZ (10 nM – 10 μM) enhanced concentration-dependent PR of rat isolated aortic segments with EC_{50} values (in 10 nM) of 2.7±0.52, 7.2±0.73, 8.1±0.65, 92±11.1, 5.8±0.37, 6.8±0.39, and 8.9±0.83 for Bay K 8644, (−)-202791, (+)-PN202791, STZ, RK 30, NI 104, and NI 105 respectively.

Effects of light exposure time on responses to Bay K 8644

In tissue preparations precontracted with phenylephrine (10 nM), irradiated Bay K 8644 solutions (1 μM) caused transient relaxations followed by contractile responses. The intensity of these relaxation and contractile responses were dependent upon the length of time the Bay K 8644 solutions had been irradiated. Thus, increasing the length of light exposure enhanced the direct relaxation response and reduced the contractile response to Bay K 8644 (Figure 2).

Effects of xanthine/xanthine oxidase on DHPs and STZ-induced photorelaxations and NO induced relaxation

Bay K 8644 (0.1 μM), PN 202791 (1 μM) enantiomeric pairs and STZ (8 μM) enhanced the intrinsic PR (the light induced relaxation) in phenylephrine (10 nM) precontracted tissues. NO (0.1 μM) also relaxed tissues precontracted with phenylephrine. The concentration of each compound which enhanced the PR or relaxed the tissues by 65±5% was chosen as the reference concentration to study the effects of X/XO. In the presence of X/XO (2 mM/10 mu ml^{-1}) the magnitude of the PR induced by DHPs and STZ as well as NO-induced relaxations was significantly reduced. Furthermore, the relaxation induced by Bay K 8644 after irradiation for 90 min was reduced to an equivalent extent by X/XO as was the PR induced by Bay K 8644 (Table 2).

Generation and detection of NO from DHPs and STZ

A concentration-response curve for NO generation from Bay K 8644 is presented in Figure 3. Comparative data from six DHPs and STZ shows NO generation could not be detected from nifedipine, nimodipine, amlodipine or felodipine; these same DHPs also failed to induce PR in precontracted rat aortic preparations. Under identical experimental conditions and equimolar concentrations (10^{-4} M) STZ, Bay K 8644 and PN 202791 released comparable amounts of NO. STZ released 40.3 ± 5.9 nmol NO min^{-1}, Bay K 8644 released 25.0 ± 2.0 nmol NO min^{-1} and PN 202791 released 30.5 ± 1.5 nmol NO min^{-1} ($n=6$).

Discussion

Induction of PR by Bay K 8644 in vascular tissue was first reported in 1985 (Mikkelsen *et al.*, 1985) and we, and others, have confirmed and extended these observations (Golenhofen *et al.*, 1990; Triggle & Bieger, 1990; Triggle *et al.*, 1991; Chen & Gillis, 1992; Baik *et al.*, 1994). Bay K 8644 and other DHPs can also promote PR in the rat thoracic aorta and the ability to promote PR is unrelated to antagonist activity at the L-type calcium channel (Triggle & Bieger, 1990). Although Bauer & Fung (1994) have recently demonstrated that high concentrations of Bay K 8644 release NO, it has not been clearly shown that the extent of NO release is sufficient to produce PR. We have previously reported (Triggle *et al.*, 1991) that certain analogues of Bay K 8644 are markedly potent in inducing PR with the threshold for initiation of PR being approximately 1 – 5 nmol. In contrast, concentrations of at least 100 μM Bay K 8644 seem to be required in order to detect NO release (Bauer & Fung, 1994). Based upon our quantification of NO release from irradiated Bay K 8644 solutions, the threshold for de-

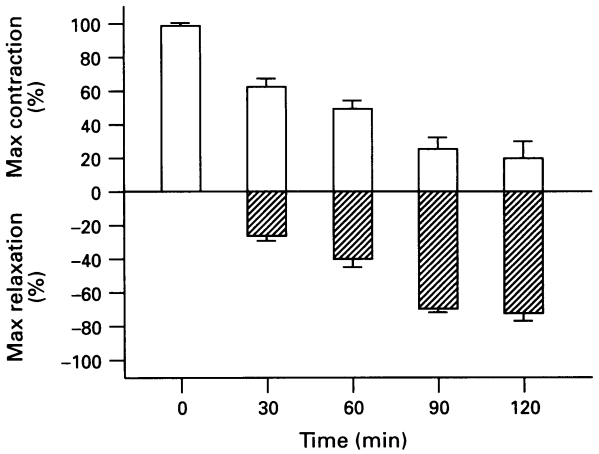


Figure 2 Effects of various irradiation periods on a 1 μM Bay K 8644 solution to induce either contraction or relaxation of aortic ring preparation partially contracted with phenylephrine. Data plotted in each column are the mean±s.e.mean ($n=5$).

Table 2 Inhibition of photorelaxation and relaxation in rat thoracic aorta with X/XO

Compounds	*Conc.* (μM)	*PR or relaxation as % of contraction*	*X/XO* (2 mM/10 mu ml^{-1}) *A*	*B*	n
Bay K 8644	0.1	72.7±4.5	64.0±3.6	40.6±5.3	8
Bay K 8644*	0.1	70.6±5.7	60.9±5.4	37.0±7.6	6
(+)-PN202791	1.0	54.2±3.3	19.3±3.5	10.5±1.5	6
(–)-PN202791	1.0	58.5±4.0	20.0±3.5	13.8±2.3	6
STZ	8.0	57.8±1.8	19.0±2.4	12.6±1.5	8
NO	0.1	66.5±3.7	6.0±1.3	6.0±1.3	8

To study the effect of O_2^- on relaxation induced by NO or photorelaxation induced by DHPs or STZ, O_2^- was generated by incubation of X (2 mM) plus XO (10 mu ml^{-1}) in Krebs solution for 1 h. After adding X/XO and raising tone, relaxation or photorelaxation was measured at 5 (a) and 25 (b) min. The results are reported as the means±s.e.mean of (n) experiments. *Indicates that Bay K 8644 solution was irradiated for 90 min.

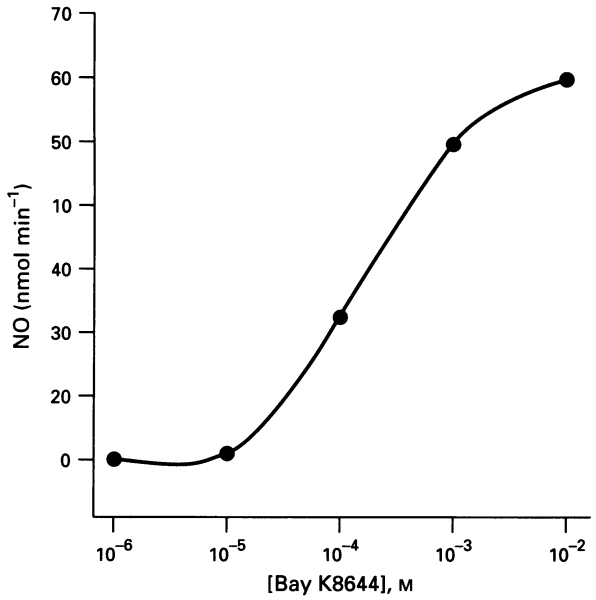


Figure 3 Bay K 8644 concentration-response curve; nmol NO generated per min.

tection of NO release, with our flow detection technique is $\geqslant$ 10 μM Bay K 8644. Most 1,4-DHPs, however, are lipophilic and [^{3}H]-nitrendipine has been reported to accumulate in cardiac cells with a cell to medium ratio of 120:1 (Lüllmann & Mohr, 1987). Thus, the accumulation of Bay K 8644 and other 3-NO_2 DHPs in rat aortic vascular smooth muscle cells would provide an intracellular source of photoreleasable NO to activate the soluble guanylyl cyclase and may explain the higher potency of these compounds versus STZ.

Irradiated Bay K 8644 solutions showed a decreased ability to induce PR. In tissues partially contracted with phenylephrine non-irradiated solutions of Bay K 8644 induce a further increase in tone; however, following irradiation of the Bay K 8644 solutions for varying periods of time a transient relaxation was observed prior to contraction. With an increase in the period of irradiation the magnitude of the transient relaxation increased and subsequent contraction decreased. Incubation of the irradiated Bay K 8644 solution with X/XO significantly attenuated the direct relaxation induced by the irradiated solution. The ability of X/XO to inhibit the vascular relaxation effect of irradiated Bay K 8644 was equivalent to the inhibition by X/XO of Bay K 8644 supported PR. These data indicate that Bay K 8644 is photodegraded and the product(s) of photodegradation are sensitive to superoxide ions. The data also suggest, but not conclusively, that the principal photodegradation product is NO. There are reports showing that nifedipine yields two photodegradation products depending on the source of irradiation. One product, nitrophenylpyridine, is obtained after photochemical oxidation of nifedipine by u.v. light, and the other, nitrosophenylpyridine, formed upon daylight irradiation (Jakobsen *et al.*, 1979). Given the chemical similarity in the structures of Bay K 8644 and nifedipine, photodecomposition of the former might also be expected. Our data from the bioassay studies with irradiated and non-irradiated solutions support the concept of Bay K 8644 phototransformation. Similarly NO, and low concentrations of compounds that can activate endothelial nitric oxide synthase, such as acetylcholine, induce transient relaxations similar to the relaxations observed in tissues treated with irradiated Bay K 8644 solutions further supporting the hypothesis that NO released from certain molecules by irradiation provides, via the activation of soluble guanylyl cyclase, the cellular mediator of PR.

It can thus be argued that DHPs and STZ can induce PR via NO release following light exposure. Support for this conclusion is provided by our observation that PR was reduced after tissue treatment with X/XO. X/XO is a generator of superoxide anions that very effectively scavenge NO, generating peroxynitrite (Blough & Zafirou, 1985) and inhibiting DHP or STZ-induced PR. Although X/XO significantly decreased DHP and almost eliminated STZ-induced photoresponses, PR, notably that induced by DHPs, was not completely abolished. This suggests that there may be a non-NO mediated mechanism that is involved in PR. Furthermore, the potency of X/XO in attenuating PR varies markedly with different DHPs and STZ. In addition, the time scale for the inhibition of DHP and STZ induced PR by X/XO varies, and this also suggests that a single molecular action for DHP-induced PR is unlikely. The attenuation of Bay K 8644 photoactivated PR by X/XO developed much more slowly than the attenuation of PR induced by other DHPs or STZ, and a significant PR response remained to Bay K 8644 after X/XO treatment. Thus the Bay K 8644-induced PR was more persistent than that induced by STZ and (−)-PN 202791 or (+)-PN 202791. X/XO was most effective in inhibiting the NO-induced relaxation; however, the finding that X/XO significantly reduced PR induced by DHPs and STZ suggests that the release of NO from these molecules by irradiation is a major contributor to the PR response to both DHPs and STZ. Furthermore, X/XO will generate superoxide anions extracellularly and thus the resistance of the Bay K 8644 induced PR may simply reflect that Bay K 8644 has a significant intracellular site of action that also involves photodegradation to generate NO.

We have also tried to correlate the bioassay measurement of PR with analysis of NO by the chemiluminescence technique. Direct measurements of NO indicate that equimolar concentrations of DHPs and STZ release similar amounts of NO following exposure to light.

Our structure-activity studies indicate that a nitro group at the C-3 position of the dihydropyridine ring is necessary to support DHP-induced PR. That the dihydropyridine ring is essential is suggested by the lack of activity demonstrated by methyl 2,6-dimethyl-3-nitroso-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate (compound 72), wherein the dihydropyridine ring is replaced by a pyridine ring and the 3-nitro substituent is replaced by a 3-nitroso substituent. The substitution of the 3-nitro substituent of Bay K 8644 by an amido ($CONH_2$) substituent (compound 99), or the presence of a guanidino group in 5-isopropyl 3-(2-guanidinoethyl) 2,6-dimethyl-1,4-dihydro-4-(2,3-dichloro-phenyl)-pyridine-3,5-dicarboxylate (compound 102) preserved PR; however, a 3-nitrooxyethyl substituent in 3-nitrooxyethyl 5-isopropyl 2,6-dimethyl-1,4-dihydro-4-(2-trifluoromethylphenyl)-pyridine-3,5-dicarboxylate (compound 85) eliminated photorelaxant activity. The relocation of the 3 nitro group from the dihydropyridine ring to the phenyl ring (i.e., nifedipine and nimodipine) also eliminated PR activity. It is noteworthy that there is an apparent lack of stereoselectivity in the ability of DHPs to induce PR. Thus both enantiomers of Bay K 8644, and PN 202791 are capable of inducing PR and, as indicated in Figure 1 for the enantiomers of PN 202791, show no significant differences with respect to the concentration-effect relationship for PR. This suggests that the molecular target for DHPs for inducing PR does not have the properties of a classical drug receptor and further supports the hypothesis that it is the photodependent release of NO that provides the most important cellular mediator of PR.

We have recently reported (Martin-Caraballo *et al.*, 1995) that 3-NO_2 DHPs such as PN 202791 and Bay K 8644 also induce PR in the rat isolated oesophageal smooth muscle, a tissue that lacks intrinsic sensitivity to light. In the rat oesophagus the 3-NO_2 DHP photoactivated PR has fast and slow components. The former appears to result primarily from modulation of the open state of the L-calcium channel as this component of the PR is abolished by chelation of extracellular calcium, skinning of the plasmalemma or blockers of the calcium channel. The 3-NO_2 DHP photoactivated PR that we

describe for the rat aorta seems to correspond to the slow component in the rat oesophagus, in that both are insensitive to blockade of the L-calcium channel and/or depletion and chelation of extracellular calcium (Triggle & Bieger, 1990).

In conclusion, this study has demonstrated that certain DHPs, notably those possessing a 3-nitro grouping, are capable of supporting photoactivated relaxation of precontracted rat thoracic aortic tissue. The ability of DHPs, such as Bay K 8644, to support photorelaxant activity is similar to that shown by the diabetogenic agent STZ. Of the photoactive compounds tested all could be shown to release NO when exposed to polychromatic light and, furthermore, there was an association between the loss of photorelaxant activity of DHPs and STZ and the length of exposure to superoxide anion generating solutions. These data suggest strongly that the photoactivated release of NO underlies the PR response. However, we have previously reported the remarkable potency of certain analogues of Bay K 8644 (Triggle *et al.*, 1991), and furthermore in the present study, the PR-inducing activity of Bay K 8644, unlike STZ, was partially resistant to a superoxide anion generating solution. These data suggest that in addition to NO release, the PR activity of DHPs may also depend on, as yet, unknown cellular effects on the contractile activity of vascular tissue.

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