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European Journal of Pharmacology 459 (2003) 263–267
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Mediation of nitric oxide from photosensitive stores in the photorelaxation of the rabbit corpus cavernosum

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Received 25 July 2002; received in revised form 26 November 2002; accepted 29 November 2002

Abstract

Isolated rabbit corpus cavernosum relaxed in response to ultraviolet (UV) light (365 nm). The UV light-induced relaxation (photorelaxation) was diminished on repeated UV irradiation from 30.5 ± 4.0% (the first photorelaxation) to 15.5 ± 2.7% (the last photorelaxation). Hydroxocobolamine of 100 μM and hemoglobin (Hb) of 10 μM, which are nitric oxide (NO) scavengers, and 10 μM 1*H*-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), a soluble guanylyl cyclase inhibitor, markedly reduced photorelaxation. However, 300 μM 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide (carboxy-PTIO) failed to inhibit photorelaxation. $NaNO_2$ and N^G-nitro-L-arginine (L-NA) but not 3-nitro-L-tyrosine (3-NT) were found to be photosensitive in that these compounds are photolysed to release NO, as demonstrated by use of an amperometric NO probe; NO signals produced by 500 μM $NaNO_2$, and 500 μM L-NA were 133.3 ± 28.9 and 54.4 ± 10.4 pA, respectively. Not 3-NT but the other compounds (all 200 μM) also enhanced photorelaxation of the cavernosal tissue. Based on these findings, the substance, which mediates photorelaxation, could be NO released from putative stores in the rabbit corpus cavernosum, and L-NA as well as $NaNO_2$ but not 3-NT produce NO under the influence of UV light.

Keywords: Corpus cavernosum; Nitric oxide (NO); 3-Nitro-L-tyrosine; Photorelaxation; Ultraviolet light

1. Introduction

Exposure of rabbit aorta (Furchgott et al., 1955) and mouse gastric fundus (Öğülener et al., 1996) to ultraviolet (UV) light results in relaxation (photorelaxation), the characteristics of which are very similar to those of the relaxation induced by nitric oxide (NO) (Furchgott et al., 1984, 1985), although hyperpolarizing factor (Charpie et al., 1994) is also suggested as one of the photo-induced relaxing factors (PIRF) (Furchgott and Jothianandan, 1991). The photorelaxation is independent of the endothelium, and the source of NO-yielding compound(s) is proposed to be in smooth muscle cells (Furchgott et al., 1984). In addition, the presence of a store of photoactivable materials has been proposed in the rabbit aorta (Venturini et al., 1993). This has recently been supported by Büyükafşar et al. (1999) in mouse gastric fundus, where endogenous NO can refill this store. In order to ascertain the finding that endogenously released NO could replenish the putative store which yields NO by the action of UV light, cavernosal tissue was chosen because it is a suitable preparation as it can be stimulated by acetylcholine and nitrergic nerve stimulation, both of which are NO-synthesizing and releasing stimuli. However, first of all, in the present work, we studied the action of UV light on the tissue, and describe some properties of photorelaxation. Furthermore, we assessed NO generation from some photosensitive compounds by use of an amperometric NO probe because there are no studies of the direct formation of NO from such compounds. We also examined whether 3-nitro-L-tyrosine (3-NT), which is a marker of peroxynitrite formation, was photosensitive since it contains an aromatic nitro group within its chemical structure which can be photolysed to release NO, as in some compounds such as a calcium channel opener, 1,4-dihydro-2,6-dimethyl-5-nitro-4-[2-(trifluoromethyl)phenyl]pyridine-3-carboxylic acid methyl ester (BAY K 8644) (Chen and Gilles, 1992).

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0014-2999/02/$ - see front matter © 2002 Published by Elsevier Science B.V.
doi:10.1016/S0014-2999(02)02858-3

2. Materials and methods

2.1. Tissue preparation

Male New Zealand rabbits weighing 2–2.5 kg were used in the study. The animals were killed by intravenous injection with thiopenthal Na (50 mg kg^{-1}) followed by exsanguination. Thereafter, the penis was removed en bloc. Cavernosal preparation was prepared as described elsewhere (Yıldırım et al., 1999). Strips of corpus cavernosum (~ 2 × 2 × 15 mm) were placed with the cavernosal sinusiods oriented to the source of UV light in an organ bath (20 ml) filled with Krebs-bicarbonate solution (in mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25, $MgSO_4$ 1.2, $KHPO_4$ 1.2, glucose 11) gassed with 95% O_2 and 5% CO_2. The pH of the solution was kept at 7.4, and the temperature was maintained at 37 °C. Each rabbit provided two to four cavernosal strips that were studied separately. Tension was recorded isometrically with a force transducer (COMMAT, Ankara, Turkey) and displayed on a Biopac acquisition system (Biopac Systems, California, USA). The preparations were allowed to equilibrate for 1 h, during which the tension was adjusted to 2 g. UV light was given from 4-W UV lamps (UVL-21, Cambridge, UK) with peak intensity at 365 nm. The lamps were placed next to the outer wall of a jacked glass incubation chamber, and the distance between UV lamp and the cavernosal strip was about 2–3 cm.

2.2. Photorelaxation experiments

After the equilibration period, the cavernosal strip was submaximally contracted with 5 μM phenylephrine. After a steady-state contraction was achieved, the tissue was irradiated with UV light (365 nm) five times for 2 min at 3-min intervals (first series). After the first series of photorelaxation, 100 μM hydroxocobolamine, 10 μM hemoglobin (Hb), 300 μM 2-(4-carboxphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide (carboxy-PTIO), 10 μM 1*H*-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), 200 μM N^G-nitro-L-arginine (L-NA), 200 μM $NaNO_2$ or 200 μM 3-nitro-L-tyrosine (3-NT) was added to the organ bath and allowed to equilibrate for 10 min. Thereafter UV light was applied for five times as in the first series. In control series, no drugs were added but time-course experiments were performed.

2.3. Determination of NO formation by UV light

In order to determine whether the compounds which were able to relax cavernosal strips on exposure to UV irradiation can be photolysed to release NO, we directly measured NO by use of ISO-NOP 200 amperometric electrodes fitted to an ISO-NO mark II nitric oxide meter (WPI, Berlin, Germany). NO signals were captured and displayed on a Biopac acquisition system. Based on the suggestion of the manufacturer, NO-sensitive electrodes were calibrated by generating nitric oxide from 50 μM $NaNO_2$ and the mixture of 0.1 M H_2SO_4 and 0.1 M KI. The probes were also checked before experimentation with NO generated from the reaction between 10 μM Angeli's Salt and 100 μM $CuSO_4$ (Büyükafşar et al., 2001).

Four compounds, i.e. $NaNO_2$ (500 μM), L-NA (500 μM), sodium nitroprusside (10 μM) and 3-NT (500 μM) were tested as to whether they can produce NO when exposed to UV light. These substances were dissolved in distilled water and passed through a glass tube (4.7-mm inside diameter, 6.1-mm outside diameter and 150-mm length). At the end of the tube, the NO probe was inserted in such a way that the perfusion fluid could flow out. A peristaltic pump (Peri star 500304, Hertfordshire, UK) was used to pump the fluid containing these substances at a constant rate (1 ml min^{-1}). Since the ISO NOP 200 NO probe is susceptible to UV light, it was protected against UV light by placing a radiation baffle between the UV lamp and the probe. The UV irradiation source was a 4-W UV lamp with a peak intensity at 365 nm, which was placed about 1 cm away from the glass tube. NO generation from the photosensitive substances was recorded for 2 min, as in the photorelaxation studies, and maximum signals were evaluated. Control series were done with distilled water containing none of those compounds.

2.4. Chemicals

L-Phenylephrine hydrochloride, hydroxocobolamine hydrochloride, sodium nitroprusside, 3-nitro-L-tyrosine, N^G-nitro-L-arginine and human hemoglobin were obtained from Sigma (St. Louis, MO, USA). 2-(4-Carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide (carboxy-PTIO) and 1*H*-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) were obtained from Tocris Cookson (Bristol, UK) and $NaNO_2$ from Merck (Darmstadt, Germany). ODQ was dissolved in dimethyl sulfoxide (DMSO) and the others in distilled water.

2.5. Analysis of results

Photorelaxation is expressed as a percentage of the phenylephrine-induced contraction and shown as mean ± S.E.M. Comparisons were made by Student's *t*-test for unpaired observation, using a computer-based program (Graph-Pad, Prism, California, USA).

3. Results

3.1. Photorelaxation

Isolated rabbit corpus cavernosum relaxed in response to UV irradiation. Initial photorelaxation markedly differed

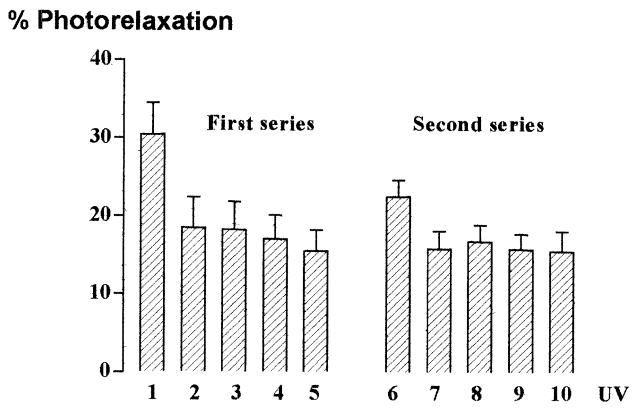

Fig. 1. Photorelaxation by UV light (365 nm) in control ($n=8$). Note the diminished relaxation to consecutive light exposure, and partial restoration of the initial photorelaxation in the second series. Relaxation is expressed as a percentage of 5 μM phenylephrine-induced tone. After a steady-state, contraction was established, the tissue was irradiated with UV light (365 nm) five times for 2 min at 3-min intervals (first series). There was 10 min between the first and the second series. Data represent means ± S.E.M. of eight observations.

from one tissue to another in that the smallest photorelaxation was 5.6% while the largest one was 230% of the active tone, i.e. in some tissues, UV light evoked a relaxation below resting tone before phenylephrine was administered. Photorelaxations reduced on repeated UV light exposure (Fig. 1). In most tissues, photorelaxation was rapid in onset and recovered quickly even though UV irradiation was still on. In the second series, initial photorelaxation was nearly restored to the corresponding control (first series) value.

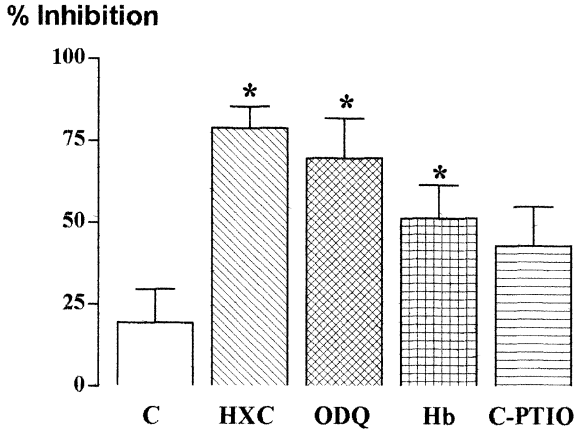

Fig. 2. Inhibition of photorelaxation by hydroxocobolamine (HXC, 100 μM, $n=7$), ODQ (10 μM, $n=5$) or Hb (10 μM, $n=8$) but not carboxy-PTIO (300 μM, $n=5$). The compounds were incubated for 10 min. Percent inhibition was calculated as follows: $1-(a/b)\times 100$. a: the percent initial photorelaxation of the second series (in the presence of the inhibitors), b: the percent initial photorelaxation of the first series (in the absence of the inhibitors). *$P<0.05$, significantly different from control, Student's t-test for unpaired observations. Data represent means ± S.E.M. of five to eight observations. C: control.

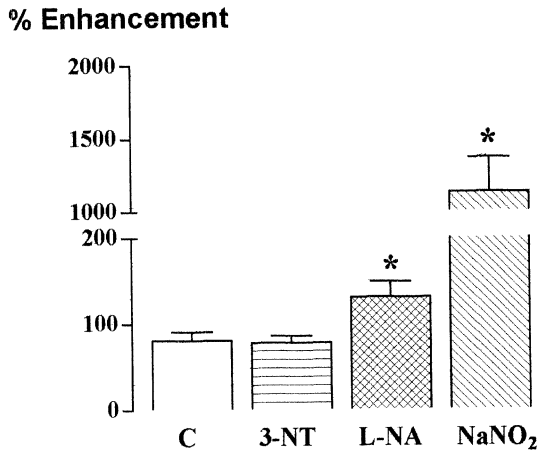

Fig. 3. Enhancement of photorelaxation with some of the nitro-containing compounds. 3-NT of 200 μM ($n=8$), 200 μM L-NA ($n=9$) or 200 μM $NaNO_2$ ($n=6$) was incubated for 10 min after the first series and then the second series was performed. Percent enhancement was calculated as follows: $a/b\times 100$. a: the percent initial photorelaxation of the second series (in the presence of the enhancing compounds), b: the percent initial photorelaxation of the first series (in the absence of the enhancing compounds). *$P<0.05$, significantly different from control, Student's t-test for unpaired observations. Data represent means ± S.E.M. of six to nine observations. C: control.

3.2. Effects of hydroxocobolamine, Hb, carboxy-PTIO, ODQ, 3-NT, L-NA, and $NaNO_2$

Hydroxocobolamine (100 μM) ($n=7$), 10 μM Hb ($n=8$) or 10 μM ODQ ($n=5$) but not 300 μM carboxy-PTIO ($n=5$) inhibited UV-elicited relaxation (Fig. 2). The inhibition by carboxy-PTIO was 43.0 ± 12.0% but it was found nonsignificant (control value was 19.4 ± 10.0%, $P>0.05$).

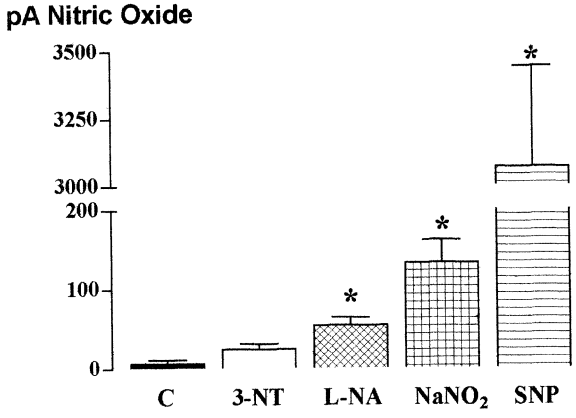

Fig. 4. Generation of NO signals from $NaNO_2$ (500 μM, $n=6$), L-NA (500 μM, $n=5$) and sodium nitroprusside (SNP, 10 μM, $n=6$) but not from 3-NT (500 μM, $n=7$) under the influence of UV light. The substances were dissolved in distilled water and passed through a glass tube, which was irradiated with UV light (365 nm). At the end of the tube, an amperometric NO probe was inserted, and continuous NO signals were captured for 2 min. *$P<0.05$, significantly different from control, Student's t-test for unpaired observations. Data represent means ± S.E.M. of five to seven observations. C: control.

Not 3-NT (200 μM, $n=8$) but L-NA (200 μM, $n=9$), and $NaNO_2$ (200 μM, $n=6$) markedly enhanced photorelaxation (Fig. 3).

3.3. Generation of NO from the photosensitive compounds in the perfusing solution

UV light produced NO signals from $NaNO_2$ (500 μM, $n=6$), L-NA (500 μM, $n=5$) and SNP (10 μM, $n=6$) but not from 3-NT (500 μM, $n=7$) (Fig. 4).

4. Discussion

In the present study, we examined the effects of UV light on the isolated rabbit corpus cavernosum. The photorelaxation phenomenon was first demonstrated by Furchgott et al. (1955) in rabbit aorta. The characteristics of photorelaxation were later investigated, and it was found that NO could be involved in the UV-elicited relaxation, based on the findings that the photorelaxation was inhibited by hemoglobin and methylene blue, and was accompanied by an increase in cGMP (Furchgott et al., 1984, 1985; Furchgott and Jothianandan, 1991). In addition to NO, a hyperpolarizing factor has been proposed as a photo-induced relaxing factor in genetically hypertensive rats (Charpie et al., 1994). In mouse, gastric fundus NO or a related species may be responsible for photorelaxation (Öğülener et al., 1996). It has been suggested that rabbit aorta may contain a depletable and replenishable store of photosensitive materials (Venturini et al., 1993). Likewise, Büyükafşar et al. (1999) have recently proposed that mouse gastric fundus may also have a store of NO-yielding photosensitive compounds which is easily depleted after consecutive UV light exposure, and they demonstrated for the first time that the store could be refilled by endogenous NO released from nitrergic nerves. Support for this finding has come from Triguero et al. (2000). They have suggested that a photo-labile, nitro-compound store is replenished by nitrergic neurotransmission in urethral smooth muscles.

Although the chemical identification of the photosensitive and NO-yielding materials is not known completely, intracellular nitrite, nitrosylated compounds and/or nitro-containing substances have been proposed inasmuch as L-NA, BAY K 8644, which contains an aromatic nitro group, nitrosothiols, iron sulfur complexes and nitrite ions are all photosensitive and generate NO or related species (Chen and Gilles, 1992; Williams, 1985; Flintney et al., 1993; Ehrreich and Furchgott, 1968). Chen and Gibes (1993) demonstrated the enhancement of photorelaxation in corpus cavernosum with methylene blue, and speculated that in addition to NO, a compound generated from methylene blue by the action of UV light might stimulate guanylyl cyclase.

In the present study, however, we used more specific tools that interfere with NO action such as hydroxocobolamine, hemoglobin and carboxy-PTIO (Li and Rand, 1999), as NO scavenging or oxidizing agents, and ODQ (Garthwaite et al., 1995) as a soluble guanylyl cyclase inhibitor. Except for carboxy-PTIO, they all inhibited photo-induced relaxation substantially, indicating the mediation of NO and soluble guanylyl cyclase in photorelaxation. Moreover, in the study, we demonstrated that $NaNO_2$, L-NA and sodium nitroprusside can indeed release NO under the influence of UV irradiation, as detected directly with the help of an NO probe.

Apart from NO, the formation of superoxide anion (O_2^-) from tissue by the action of UV light has been proposed (Matsunaga and Furchgott, 1989). NO and O_2^- react to form peroxynitrite ($ONOO^-$), which are nitrate tyrosine residues of proteins (Ischiropoulos et al., 1992). Consequently, a stable end product, nitrotyrosine, which is a marker of peroxynitrite formation, can be produced. Based on the findings that BAY K 8644 and L-NA contain a photosensitive nitro moiety, we were interested to explore whether the biologically produced nitro-compound, nitrotyrosine, can be photolysed to release NO as one of the endogenous photosensitive materials. Unfortunately, the compound is neither photosensitive to release NO nor capable of relaxing the cavernosal tissue upon exposure to UV irradiation, as confirmed in the tissue medium and by use of an amperometric NO-sensitive probe.

In conclusion, NO or a related species may be involved in the photorelaxation of the isolated rabbit corpus cavernosum. The nature of endogenous photosensitive substances still remains unclear. Nevertheless, the findings of the study may give support to the hypothesis that intracellular nitrite, nitrosylated substances and nitro-containing compounds could be the endogenous sources of NO-yielding photosensitive materials (Büyükafşar et al. 1999).

Acknowledgements

This work has been supported by the Turkish Academy of Sciences, in the framework of Young Scientist Award Program (EA-TÜBA-GEBİP/2001-1-1).

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