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Photoactivation of the nitric oxide donor SIN-1

Thomas Ullrich, Stefanie Oberle, Aida Abate, Henning Schröder*

Department of Pharmacology and Toxicology, School of Pharmacy, Martin Luther University, Wolfgang-Langenbeck-Str. 4, 06099 Halle (Saale), Germany

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Abstract This study investigates whether nitric oxide (NO) release from linsidomine (SIN-1), the active metabolite of the antianginal drug molsidomine, is light sensitive. A 9-h irradiation with polychromatic visible light increased the formation of nitrite (NO index metabolite) from SIN-1 (1 mM) by 61% as compared to control samples incubated in the dark. Under the same conditions (9-h irradiation) the concentration of oxygen decreased to 2% of control. However, oxygen consumption was substantially reduced when light was excluded (35% recovery after 9 h). These results show that irradiation with visible light markedly enhances the oxygen-dependent NO release from SIN-1. Thus, sydnonimines appear to be suitable model compounds for the development of photoactivatable NO donors.

Key words: SIN-1; Linsidomine; Sodium nitroprusside; Nitric oxide; Nitrite; Photoactivation

1. Introduction

Organic nitrates have remained useful agents for the management of myocardial ischemia and its principal symptom angina pectoris since they were introduced into clinical therapy over a hundred years ago. The cellular mechanism underlying their antianginal action involves conversion of these compounds into the nitric oxide (NO) free radical, which then stimulates the vasodilatory second messenger cyclic GMP (for review see [1]). Thus, organic nitrates have to be classified as prodrugs releasing the pharmacologically active metabolite NO. In the case of the nitric acid esters, previous studies suggest an enzymatic process to be responsible for the bioactivation of, i.e. NO release from, these compounds [2,3]. In contrast to that, sodium nitroprusside and sydnonimines have long been considered to be purely spontaneous NO donors which decompose in aqueous solutions generating NO without requiring enzymatic bioactivation or other co-factors. Recently, however, it has been shown that in vitro sodium nitroprusside releases NO only in the presence of light whereas no formation of NO was detected in the dark [4]. These observations have raised questions as to the 'spontaneous' character of NO formation from sodium nitroprusside in vivo, i.e. in vascular tissue, where light is absent [4]. Linsidomine (SIN-1), the active metabolite of molsidomine, is also considered a spontaneous donor of NO and frequently used to provide prophylaxis against anginal episodes without causing tolerance even during long-term treatment.

In the present study the effect of light on NO formation from SIN-1 is investigated and compared to the photochemical activation of sodium nitroprusside under the same conditions.

2. Materials and methods

2.1. Materials

A tungsten lamp (wolfram, 40 W) was used to generate visible, polychromatic light. By using an irradiation chamber, which was lined with aluminum foil in the interior, radiation was spread evenly (light intensity: 3500 lux). Incubations were carried out at 25°C in all-glass volumetric flasks. Flasks were wrapped with aluminum foil to exclude light and perform incubations in the dark. The temperature in the chamber and in the samples was closely monitored and remained constant during irradiation. Thus, a temperature-dependent effect on NO release from SIN-1 under the chosen conditions can be excluded. NO donors were dissolved in air saturated phosphate buffer (Sörensen, 67 mM, pH = 7.6). The final volume of irradiated solutions during assessment of NO release was 5 ml.

SIN-1 was provided by Hoechst AG (Frankfurt, Germany). Sodium nitroprusside, sulfanilamide and naphthylethylenediamine were obtained from Sigma. All other reagents were of analytical grade or were purified to HPLC purity prior to use.

2.2. Measurement of nitrite/nitrate

NO generation was measured by assessing the amount of the stable NO metabolite nitrite. Nitrite was quantitated colorimetrically via the Griess reaction as described previously [5]. 60 μl sample aliquots were incubated for 10 min with 80 μl of 1% sulfanilamide in 4 N HCl at room temperature. After adding 60 μl of 1% naphthylethylenediamine in methanol, incubation was continued for another 10 min. Nitrite concentration proportional to OD_{540} was determined using a microplate reader (BIO-TEK, EL 311s), with reference to a standard curve. The simultaneous determination of nitrite and nitrate ions was performed using an HPLC method described previously including anion exchange chromatography and photometric detection at 200 nm [6,7]. Prior to sample injection, remaining sydnonimines and their degradation products, which interfered with the measurement of nitrite/nitrate, were removed by solid phase extraction using cation exchange columns (Adsorbex SCX, Merck, Darmstadt, FRG).

2.3. Measurement of oxygen consumption

Oxygen consumption was assessed polarographically using a Clark electrode [8,9]. Air saturated phosphate buffer (Sörensen, 67 mM, pH = 7.6) was filled into a closed all-glass system and maintained at 25°C under continuous stirring. Freshly prepared solutions of NO donors were injected and oxygen consumption was recorded.

3. Results

After a 9-h incubation of SIN-1 (1 mM) in the absence of light, a concentration of 117 μM nitrite was measured with the Griess assay. Under the same conditions, irradiation with light increased nitrite formation from SIN-1 by 61% to 188 μM (not shown). Similar results were obtained with the HPLC method showing that irradiation with light led to an increased formation of both nitrite and nitrate in a solution containing SIN-1 (Fig. 1).

Sodium nitroprusside (1 mM) released 70.7 μM nitrite after

*Corresponding author. Fax: (49) (345) 552-7025.
E-mail: schroeder@pharmazie.uni-halle.de

Abbreviations: SIN-1, linsidomine (3-morpholinosydnonimine); NO, nitric oxide

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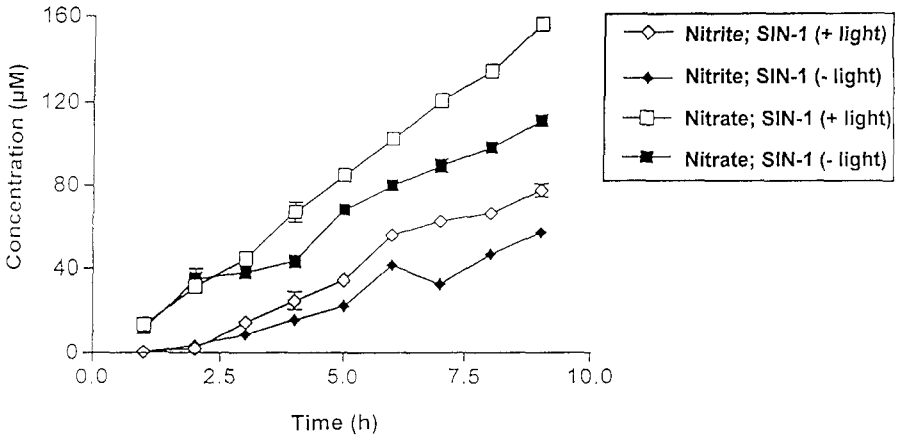

Fig. 1. Effect of irradiation with visible light on the formation of nitrite and nitrate from 1 mM SIN-1 (HPLC). The data are means ± S.E.M. of n=5 observations. Irradiated samples (+light) vs. samples kept in the dark (−light) were found to be significantly different from time point 5.0 h to time point 10.0 h ($P < 0.05$, two-tailed t-test).

a 9-h irradiation but no detectable amounts of nitrite were generated in the dark (Fig. 2).

Since NO formation from SIN-1 is an oxygen consuming process we further studied the light dependence of the oxygen consumption by SIN-1. The oxygen concentration in a 1 mM solution of SIN-1 decreased to 2% of control during a 9-h exposure to light (100% equals 250 µM oxygen at time point zero, Fig. 3). Under the same conditions, oxygen consumption was substantially reduced when light was excluded (35% recovery after 9 h, Fig. 3).

4. Discussion

The present study demonstrates that NO release from SIN-1 in vitro is markedly enhanced by visible light. In aqueous oxygen containing solutions NO is quickly oxidized to nitrite and nitrate (via hydrolysis of intermediate N_2O_4), which can both be used as stable index metabolites of NO [5,8]. In the case of SIN-1, nitrate formation may also involve intermediate generation of peroxynitrite from NO and superoxide [8]. Using the colorimetric Griess reaction we found a light-induced increase in nitrite formation from SIN-1. Similar results were obtained with an HPLC method showing that irradiation with light led to an increased formation of both nitrite and nitrate in a solution containing SIN-1. These results demonstrate that the light-induced increase in nitrite ions measured by the Griess reaction is actually due to increased generation of NO from SIN-1 and not caused by changes in the subsequent oxidation of NO (nitrite/nitrate ratio) such as increased nitrite and reduced nitrate formation. This is supported by our observation that oxygen consumption, which triggers NO release from SIN-1 [8,9], was also enhanced in the presence of light. Interestingly, oxygen concentration during SIN-1 breakdown fell to close to zero after about 5 h whereas NO formation continued. However, this apparent discrepancy may be explained by recent findings that after the consumption of oxygen, NO formation within a SIN-1 solution continues either via reaction of remaining unmetabolized SIN-1 with oxidants other than oxygen or from intermediate nitrogen oxide species with a longer half-life than NO itself [8].

Sodium nitroprusside, another spontaneous NO donor, was found to release no nitrite at all when incubated in the dark, which is in line with a previous study [4]. In contrast, SIN-1 is able to generate NO in the dark but appears to share with sodium nitroprusside the sensitivity to photoactivation. Although photolysis itself probably makes no physiological contribution, a modulation by visible light of NO release from NO donors has been linked to enzymatic bioactivation

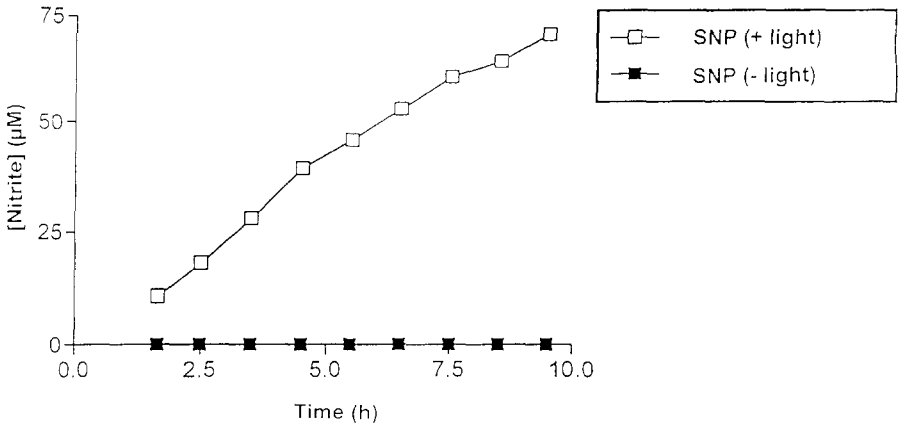

Fig. 2. Effect of irradiation with visible light on the formation of nitrite from 1 mM sodium nitroprusside (Griess reaction). The data are means ± S.E.M. of n = 5 observations. Irradiated samples (+light) vs. samples kept in the dark (−light) were found to be significantly different for every time point shown ($P < 0.05$, two-tailed t-test).

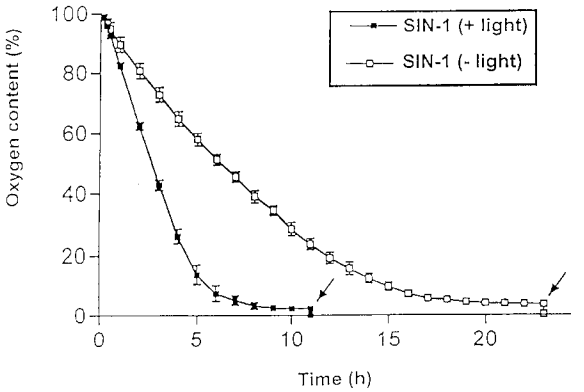

Fig. 3. Effect of irradiation with visible light on oxygen consumption by 1 mM SIN-1. The data are means ± S.E.M. of $n = 5$ observations. Arrows mark the addition of sodium dithionite (10 mM) in order to stop the reaction and control for remaining oxygen content (< 3%) under equilibrium conditions. Irradiated samples (+light) vs. samples kept in the dark (−light) were found to be significantly different from time point 2 h ($P < 0.05$, two-tailed t-test).

of the respective compound in vivo, i.e. in vitro visible light may substitute for the reduction of activation energy during enzymatic catalysis [4,10]. Thus, our findings suggest that in addition to spontaneous NO release, enzymatic or other energy yielding processes may contribute to bioactivation of SIN-1 in vivo.

Interestingly, Venturini and co-workers [10] reported a photoactivatable store of endogenous NO in vascular smooth muscle suggesting similarities in the release mechanism of NO containing drugs and physiological NO stores (endothelium-derived relaxing factor). Iron-sulfur cluster nitrosyls and other NO containing compounds have also been shown to release NO via photoactivation [11,12]. According to our findings, SIN-1 has to be added to the list of photosensitive NO donors. Photosensitivity of NO donors developed in the future may open up the possibility to confine NO generation from these compounds and subsequent pharmacological effects (e.g. vasodilation) to distinct organs or tissues by exposing the latter to laser light or other focused energy sources. Photosensitive NO generation could also be an approach to exploit the cytotoxic effects of NO at high concentrations, e.g. in tumor therapy, and to prevent or limit NO induced damage to surrounding healthy tissue. Thus, our results may explain very recent findings by Wood et al. [13] demonstrating that SIN-1 given immediately before irradiation with X-rays increases tumor cell killing in mice 2–4-fold over that for X-rays alone.

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