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European Journal of Pharmacology 450 (2002) 267–275

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A putative role for *S*-nitrosoglutathione as the source of nitric oxide in photorelaxation of the mouse gastric fundus

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Received 28 March 2002; received in revised form 4 July 2002; accepted 9 July 2002

Abstract

Mouse gastric fundus strips were relaxed by ultraviolet light (UV) irradiation, exogenous nitric oxide (NO), isoproterenol, *S*-nitrosoglutathione, *S*-nitroso-L-cysteine and *S*-nitroso-*N*-acetyl-penicillamine. Glutathione did not affect relaxations in response to UV irradiation, exogenous NO and isoproterenol while inhibiting that with *S*-nitrosoglutathione. L-Cysteine inhibited responses to UV irradiation and exogenous NO, but not in the presence of exogenous Cu^{2+}/Zn^{2+} superoxide dismutase. However, L-cysteine alone or in combination with Cu^{2+}/Zn^{2+} superoxide dismutase did not affect the relaxations in response to *S*-nitroso-L-cysteine. Ethacrynic acid and diamide inhibited photorelaxations but not the relaxations with exogenous NO and isoproterenol. This inhibition was prevented by glutathione, but not by L-cysteine. *S*-nitrosoglutathione-induced relaxations were abolished by diamide and ethacrynic acid, whereas responses to *S*-nitroso-L-cysteine and *S*-nitroso-*N*-acetyl-penicillamine were only inhibited by ethacrynic acid. These results suggest that *S*-nitrosoglutathione may, at least in part, be the putative *S*-nitrosothiol, which is converted to NO in response to UV irradiation in mouse gastric fundus strips.

Keywords: Gastric fundus; (Mouse); Photorelaxation; Nitric oxide (NO); *S*-nitrosothiol; Thiol modulator; Glutathione

1. Introduction

In 1955, Furchgott et al. demonstrated that isolated mammalian arteries in a state of active tone contraction relaxed when exposed to ultraviolet light (UV). Three decades later, the photorelaxation of vascular smooth muscle was shown to be independent of endothelium, inhibited by haemoglobin and methylene blue, and accompanied by an increase in guanosine 3′-5′ cyclic monophosphate (cGMP) (Furchgott et al., 1985). In addition, Matsunaga and Furchgott (1989) indicated that photorelaxation might also be due to endogenous nitric oxide (NO) liberated from a photodegradable molecular "store" of NO contained within the vessel wall. Subsequently, this hypothesis was supported by Venturini et al. (1993), who suggested a light-activated, depletable and replenishable NO-yielding store as being responsible for the photorelaxation of vascular smooth muscle in the isolated rabbit aortic strips. A similar conclusion was reached by Kubaszewski et al. (1994), who showed directly the release of NO via UV light by using a porphyrinic sensor in vascular smooth muscle. These authors' observations that repeated treatments with UV light cause diminished vascular smooth muscle relaxation and NO release indicate that UV light causes the release of NO from a depletable store.

In our previous study (Ögülener et al., 1996), UV light caused relaxation in isolated mouse gastric fundus strips. The photorelaxation was inhibited by haemoglobin, hydroxocobalamin, $FeSO_4$ and methylene blue, whereas N^G-monomethyl-L-arginine had no effect. These observations were interpreted as indicating that release of NO by UV light causes photorelaxation. Recently, Büyükavşar et al. (1999) showed that there could be a store of photosensitive compounds yielding NO in smooth muscle strips of the mouse gastric fundus. However, the origin and anatomic localization of the NO store are not known.

There are several candidates for the source of NO, e.g. iron–sulphur complexes, which are photolysed to release NO (Flitney et al., 1993), and thiol groups, which can react with NO in order to form *S*-nitrosothiols (Stamler et al., 1992; Megson et al., 1995; Lovren and Triggle, 1998). NO

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PII: S0014-2999(02)02098-8

is an important mediator of smooth muscle relaxation in the peripheral nervous system (Bult et al., 1990; Rand and Li, 1995); however, the short half-life of NO which may limit the efficacy and duration of its biological activity (Wood and Gartwaite, 1994) leads to the suggestion that NO might bind intracellularly to a carrier molecule, resulting in a more stable NO-containing compound from which NO is released at its site of action. It has been proposed that low molecular weight thiols such as glutathione and cysteine, which react with NO or oxides of nitrogen to form *S*-nitrosothiols, are likely candidates for such a NO-carrier molecule (Ignarro et al., 1981); these thiols are abundantly present in cells (Gow et al., 1997). Endogenous *S*-nitrosothiols may act as intermediates in the storage and/or transport of NO and consequently enhance the biological potency of NO in vascular (Myers et al., 1990; Stamler et al., 1992; Gaston et al., 1994) and nonvascular smooth muscle (Kerr et al., 1992). The biological effects of these compounds is generally attributed to the activity of NO released on homolytic decomposition of the S–NO bond (Butler and Williams, 1993). Also, *S*-nitrosothiols can decompose photochemically to liberate NO and the corresponding disulfide (Williams, 1983; Sexton et al., 1994; Singh et al., 1995; Singh et al., 1996a,b).

Assuming the existence of NO stores in gastric fundus, we investigated whether the source of NO in photorelaxation is *S*-nitrosothiols in the mouse gastric fundus. For this purpose, we compared the effects of glutathione and L-cysteine, low molecular weight thiols, and ethacrynic acid and diamide, thiol modulators, on the relaxations in response to UV irradiation and *S*-nitrosothiols, *S*-nitrosoglutathione, *S*-nitroso-L-cysteine and *S*-nitroso-*N*-acetyl-penicillamine.

2. Materials and methods

2.1. Tissue preparation

Swiss albino mice of either sex, weighing 25–30 g, were used in these experiments. They were fasted for 24 h with free access to water, then killed by stunning and cervical dislocation. The stomach was removed and longitudinal muscle strips (approximately 10 mm long and 2 mm wide) were mounted in 10-ml organ baths filled with Tyrode solution (in mM): NaCl 136.75, KCl 2.68, $CaCl_2$ 1.80, $MgCl_2 \cdot 6H_2O$ 0.95, $NaH_2PO_4 \cdot 2H_2O$ 0.4166, $NaHCO_3$ 11.904, glucose 5.05. The bath medium was maintained at 37 °C and gassed with a mixture of 95% O_2 and 5% CO_2. Muscle strips were allowed to equilibrate for a period of 60 min, during which the medium was changed every 15 min. Over the first 30 min of incubation, the strips were stretched to obtain an initial tension of 0.5 g and remained approximately at this level throughout the experiments without addition of exogenous contractile agonist. Changes in muscle length were recorded isometrically via an isometric transducer (Ugo Basile 7006, Varese, Italy) connected to an ink-writer (Ugo Basile "Gemini" 7070).

2.2. Light source

The radiation source for photorelaxation was a 6-W ultraviolet (UV) lamp with peak intensity at 366 nm (VL 6 LC; Vilber Lourmat, Cedex, France). The UV lamp was placed next to the outer wall of a jacketed glass incubation chamber. The distance from the lamp to the preparation during irradiation was about 3 cm. The UV lamp, organ bath and solution flasks were wrapped with aluminum foil to block out light.

2.3. Experimental protocols

Once a stable basal tone was obtained, two series of control relaxant responses were obtained according to one of two experimental protocols: (1) UV irradiation (60 s), exogenous NO (10 μM; administered as acidified $NaNO_2$) or isoproterenol (5 nM) were applied without rinsing the tissue between each individual application to a single tissue. (2) The concentration–response curves for *S*-nitrosothiols, *S*-nitrosoglutathione (0.1, 0.5 and 1 μM), *S*-nitroso-L-cysteine (0.01, 0.05 and 0.1 μM) and *S*-nitroso-*N*-acetyl-penicillamine (0.1, 0.5 and 1 μM), which were obtained in separate tissues.

After the relaxant responses had been obtained, the tissues were rinsed and incubated for at least 30 min and the second series of responses were recorded in the same manner. At the end of the experimental protocol, sodium nitroprusside (10 μM) was added to the organ bath to achieve maximal relaxation.

In the first series of experiments, the effects of the exogenous glutathione and L-cysteine, low molecular weight thiols and sources of free sulphydryl groups in mammalian tissue (Jocelyn, 1972), were investigated on the relaxant responses to UV irradiation, exogenous NO and isoproterenol. Also, the effects of glutathione (10 and 50 μM) and L-cysteine (10 and 50 μM) were examined on the response to *S*-nitrosoglutathione and *S*-nitroso-L-cysteine, respectively. After the first control responses were obtained, glutathione and L-cysteine were added and UV irradiation and the above-mentioned relaxant substances were applied for the second time. The tissue was incubated with glutathione and L-cysteine for 30 min. Furthermore, to clarify whether the action of L-cysteine was due to generation of superoxide anions, the influence of exogenous Cu^{2+}/Zn^{2+} superoxide dismutase (10 U/ml) alone or in combination with L-cysteine (10 and 50 μM) was studied on the relaxant responses to UV irradiation, exogenous NO, isoproterenol and *S*-nitroso-L-cysteine. Cu^{2+}/Zn^{2+} superoxide dismutase was added 30 min before UV irradiation and relaxant agents were applied.

In a second series of experiments, the effects of thiol-modulating agents, ethacrynic acid, a nonspecific sul-

phydryl alkylator (Li et al., 1994; 10 and 50 μM), and diamide, an alkylating agent that oxidizes protein sulphydryl groups and depletes intracellular glutathione (Murphy et al., 1991; 10 and 50 μM), were investigated on relaxations induced by UV irradiation, exogenous NO, isoproterenol and to *S*-nitrosothiols, *S*-nitrosoglutathione, *S*-nitroso-L-cysteine and *S*-nitroso-*N*-acetyl-penicillamine. The tissue was incubated with ethacrynic acid and diamide for 30 min.

In a third series of experiments, the effects of glutathione (10 μM) plus ethacrynic acid (50 μM), glutathione (10 μM) plus diamide (5 μM), and L-cysteine (50 μM) in the presence of exogenous Cu^{2+}/Zn^{2+} superoxide dismutase (10 U/ml) plus ethacrynic acid (50 μM) were investigated on the relaxant responses to UV irradiation, exogenous NO and isoproterenol.

In addition, the effect of 1*H*-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ; 10 μM), a selective inhibitor of soluble guanylate cyclase, was investigated on relaxations in response to UV irradiation, exogenous NO and isoproterenol. None of the drugs used in this investigation influenced the basal active tone of the tissue under study.

2.4. Drugs and solutions

Diamide, ethacrynic acid, glutathione, 1*H*-[1,2,4]oxadiazolo[4,3-a]quin-oxalin-1-one, isoproterenol, L-cysteine, *S*-nitrosoglutathione, *S*-nitroso-*N*-acetyl-D,L-penicillamine and Cu^{2+}/Zn^{2+} superoxide dismutase were obtained from Sigma (St. Louis, MO, USA). Acidified sodium nitrite, which was used as exogenous NO source, was obtained by diluting sodium nitrite in deaerated water acidified to pH 2 with HCl, was stored at −4 °C and used in its original concentration as 10 μM (Cocks and Angus, 1990). Stock solutions of *S*-nitrosocysteine were prepared with equimolar amounts of sodium nitrite and L-cysteine under acidic conditions (pH 2) according to the method of Field et al. (1978) and kept at −20 °C. Stock solutions of *S*-nitrosocysteine and acidified sodium nitrite were prepared freshly on the day of experimentation. All drugs were dissolved in distilled water except ODQ and ethacrynic acid, which were dissolved in dimethylsulphoxide; the final concentration of this solvent showed no biological effects.

2.5. Presentation of results and statistical analysis

Relaxations were expressed as percentages of the relaxation induced by 10 μM sodium nitroprusside at the end of the experiment. However, the magnitude of the relaxations varied between experimental groups. Therefore, the relaxations obtained from the second curve have been expressed as percentages of the relaxations in the first response curve for each individual stimulation in order to avoid the differences in responsiveness between experimental groups. The results were expressed as means ± S.E.M. and *n* refers to the number of animals used for each experiment. Differences in results between tissues were tested by analysis of variance (ANOVA) and *t*-test corrected for multiple comparisons (Bonferroni correction). *P* values less than 0.05 were considered to be significant.

3. Results

3.1. Relaxant responses to UV irradiation, exogenous NO, isoproterenol and S-nitrosothiols

Gastric fundus strips of mice were relaxed in a reversible manner by UV irradiation (366 nm, 60 s). This photorelaxation was fast and transient; the relaxation began after a 5- to 10-s delay and was maximal by 60 s. After the light was switched off, the tone of strips returned to its initial level within 2–3 min. Bolus injection of exogenous NO (10 μM) caused a fast and transient relaxation while the relaxation induced by isoproterenol (5 nM) was slow in onset and was sustained ($n=6$, Fig. 1A).

The *S*-nitrosothiols, *S*-nitrosoglutathione (0.1, 0.5 and 1 μM), *S*-nitroso-L-cysteine (0.01, 0.05 and 0.1 μM) and *S*-nitroso-*N*-acetyl-penicillamine (0.1, 0.5 and 1 μM) relaxed the muscle strips of mouse gastric fundus ($n=6$). *S*-nitrosoglutathione, *S*-nitroso-L-cysteine and *S*-nitroso-*N*-acetyl-penicillamine, in concentrations of 0.1, 0.01 and 0.1, respectively, did not induce relaxations, whereas the higher concentrations of *S*-nitrosoglutathione (0.5 and 1 μM), *S*-nitroso-L-cysteine (0.05 and 0.1 μM) and *S*-nitroso-*N*-acetyl-penicillamine (0.5 and 1 μM) induced concentration-

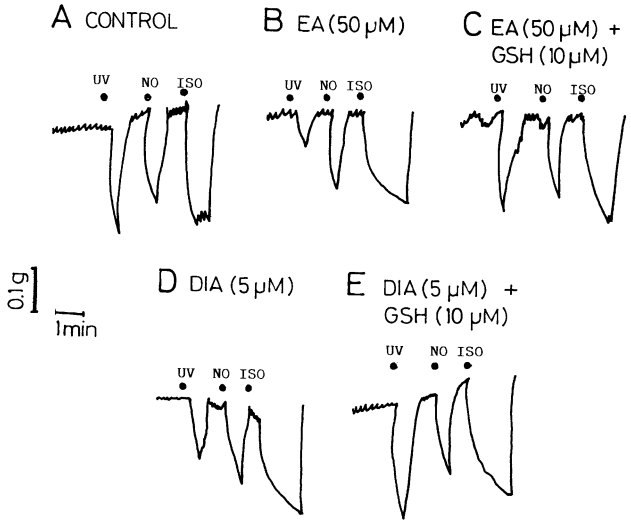

Fig. 1. Representative traces showing (A) the relaxations in response to UV irradiation (UV; 366 nm, 60 s), exogenous NO (NO; 10 μM) and isoproterenol (ISO; 5 nM); (B) the effects of ethacrynic acid (EA; 50 μM); (C) ethacrynic acid (EA; 50 μM) plus glutathione (GSH; 10 μM); (D) diamide (DIA; 5 μM); and (E) diamide (DIA; 5 μM) plus glutathione (GSH; 10 μM) on the relaxations to UV irradiation (366 nm, 60 s), exogenous NO (10 μM) and isoproterenol (5 nM) in the longitudinal strips of the mouse gastric fundus.

dependent relaxations. Data for *S*-nitrosothiols induced responses are not shown.

3.2. Effect of ODQ on relaxations induced by UV irradiation, exogenous NO, isoproterenol

ODQ (10 μM), a selective inhibitor of soluble guanylate cyclase, significantly inhibited both the photorelaxation and the relaxation in response to exogenous NO (10 μM), but not those due to isoproterenol (5 nM; data not shown).

3.3. Effect of glutathione and L-cysteine on relaxations induced by UV irradiation, exogenous NO, isoproterenol and S-nitrosothiols

To investigate the involvement of thiol groups in the mechanism of photorelaxation, the effects of exogenous free thiols, glutathione and L-cysteine were studied on the relaxant response to UV irradiation, exogenous NO, isoproterenol and *S*-nitrosothiols. Pretreatment with glutathione at 10 and 50 μM concentrations did not affect the relaxations due to UV irradiation, exogenous NO (10 μM) and isoproterenol (5 nM; $n=6$). Also, 10 μM glutathione did not influence the relaxation due to *S*-nitrosoglutathione (0.1–1 μM) whereas 50 μM glutathione significantly inhibited the relaxations induced by *S*-nitrosoglutathione ($n=6$). L-Cysteine, the other exogenous thiol, in a concentration of 10 μM, induced a significant inhibition of photorelaxation but did not affect relaxation induced by isoproterenol (5 nM; $n=6$). Also, L-cysteine tended to decrease the relaxations due to exogenous NO. To ascertain whether the inhibitory effect of L-cysteine on photorelaxation and exogenous NO was due to generation of superoxide anions, the influence of exogenous Cu^{2+}/Zn^{2+} superoxide dismutase (10 U/ml) per se or plus L-cysteine was studied on the relaxant response to UV irradiation, exogenous NO and isoproterenol. Cu^{2+}/Zn^{2+} superoxide dismutase (10 U/ml) per se did not significantly influence the photorelaxation and relaxations due to exogenous NO (10 μM), isoproterenol (5 μM) and *S*-nitroso-L-cysteine (0.01–0.1 μM), whereas the inhibitory effect of L-cysteine (10 μM) on the photorelaxation and exogenous NO was prevented by addition of Cu^{2+}/Zn^{2+} superoxide dismutase (10 U/ml; $n=6$). In addition, when a higher concentration of L-cysteine (50 μM) was added in the presence of Cu^{2+}/Zn^{2+} superoxide dismutase (10 U/ml), the relaxant responses to UV irradiation, exogenous NO and isoproterenol were no different from those of the control groups ($n=6$). Also, L-cysteine (10 μM) alone or L-cysteine (10 and 50 μM) plus Cu^{2+}/Zn^{2+} superoxide dismutase (10 U/ml) did not affect the relaxations to *S*-nitroso-L-cysteine (0.01–0.1 μM; $n=6$). The data are summarized in Table 1.

3.4. Effect of thiol modulators on relaxations induced by UV irradiation, exogenous NO, isoproterenol and S-nitrosothiols

To investigate the involvement of *S*-nitrosothiols in the mechanism of photorelaxation, the effects of thiol modulators, ethacrynic acid and diamide, were studied on the relaxant response to UV irradiation, exogenous NO, isoproterenol and *S*-nitrosothiols. Ethacrynic acid, a nonspecific thiol alkylator, (10 and 50 μM), after an incubation period of 30 min, significantly inhibited the photorelaxation in a concentration-dependent manner without affecting the basal tone ($n=6$; Figs. 1B and 2). The inhibitory effect of ethacrynic acid (50 μM) on the photorelaxation was prevented by addition of glutathione (10 μM; $n=6$; Figs. 1C and 2), but not by addition of L-cysteine (50 μM; $n=6$; Fig. 2). In contrast to photorelaxation, the relaxations due to exogenous NO (10 μM) and isoproterenol (5 nM) were not affected by ethacrynic acid (10 and 50 μM; Figs. 1B and 2). Furthermore, ethacrynic acid, in a concentration of 10 μM, significantly inhibited relaxations in response to *S*-nitrosoglutathione (0.1–1 μM), and a higher concentration of

Table 1
Effect of glutathione (GSH) and L-cysteine (L-CYS) on relaxations to UV irradiation, exogenous NO, isoproterenol (ISO), *S*-nitrosoglutathione (GSNO) and *S*-nitrosocysteine (CysNO)

	UV irradiation	NO (10 μM)	ISO (5 nM)	GSNO		CysNO	
				(0.5 μM)	(1 μM)	(0.05 μM)	(0.1 μM)
Control	98 ± 2	99 ± 3	102 ± 4	100 ± 5	100 ± 4		
GSH (10 μM)	99 ± 3	94 ± 4	104 ± 5	93 ± 4	103 ± 4	–	–
GSH (50 μM)	81 ± 4	97 ± 2	110 ± 4	11 ± 5[a]	19 ± 7[a]	–	–
Control	98 ± 2	99 ± 3	102 ± 4	–	–	103 ± 5	98 ± 2
L-CYS (10 μM)	59 ± 4[a]	77 ± 2	112 ± 6	–	–	85 ± 5	102 ± 5
SOD (10 U/ml)	101 ± 6	98 ± 4	102 ± 6	–	–	101 ± 6	101 ± 6
L-CYS (10 μM) + SOD (10 U/ml)	97 ± 6[b]	97 ± 2	106 ± 7	–	–	101 ± 6	100 ± 6
L-CYS (50 μM) + SOD (10 U/ml)	88 ± 3	96 ± 2	100 ± 4	–	–	100 ± 6	99 ± 5

Data are expressed as means ± S.E.M. ($n=6$).
[a] $P<0.05$ significantly different from control, one-way ANOVA followed by Bonferroni multiple comparison *t*-test.
[b] $P<0.05$ significantly different from L-cysteine, one-way ANOVA followed by Bonferroni multiple comparison *t*-test.

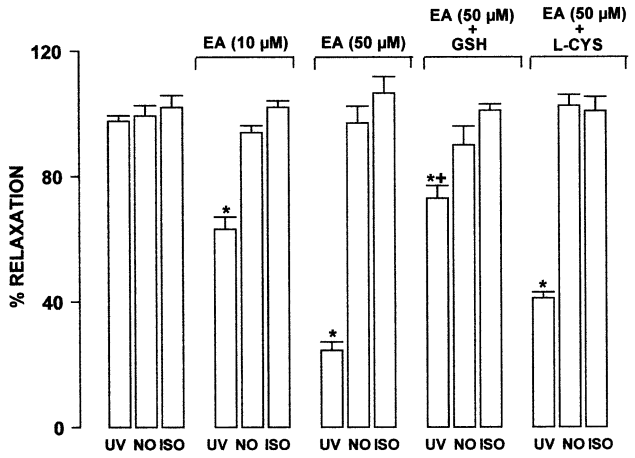

Fig. 2. Effects of ethacrynic acid (EA; 10 and 50 μM), ethacrynic acid (50 μM) plus glutathione (GSH; 10 μM) and ethacrynic acid (50 μM) plus L-cysteine (10 μM) on the relaxations due to UV irradiation (366 nm, 60 s), exogenous NO (10 μM) and isoproterenol (5 nM) in the longitudinal strips of the mouse gastric fundus. Values are means ± S.E.M. from $n=6$. * $P<0.05$ significantly different from control; $^{+}P<0.05$ significantly different from ethacrynic acid (50 μM), one-way ANOVA followed by Bonferroni multiple comparison t-test.

ethacrynic acid (50 μM) totally abolished *S*-nitrosoglutathione-induced relaxation ($n=6$, Fig. 3). On the other hand, 10 μM ethacrynic acid did not affect relaxations due to *S*-nitroso-L-cysteine (0.01–0.1 μM) and *S*-nitroso-*N*-acetyl-penicillamine (0.1–1 μM), but a higher concentration of ethacrynic acid (50 μM) significantly reduced relaxations induced by *S*-nitroso-L-cysteine and *S*-nitroso-*N*-acetyl-penicillamine ($n=6$, Figs. 4 and 5). However, the inhibition of *S*-nitroso-L-cysteine and *S*-nitrosoglutathione responses by ethacrynic acid was less than that of the *S*-nitrosoglutathione response.

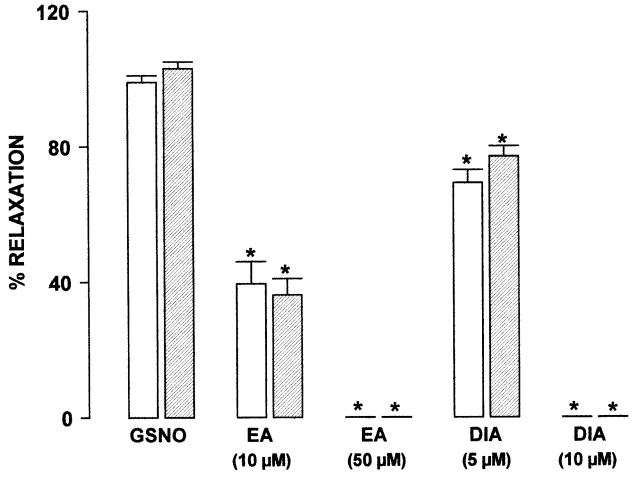

Fig. 3. Effects of ethacrynic acid (EA; 10 and 50 μM) and diamide (DIA; 5 and 10 μM) on the relaxations induced by 0.5 μM (open bar) and 1 μM (hatched bar) *S*-nitrosoglutathione (GSNO) in the longitudinal strips of the mouse gastric fundus. Values are means ± S.E.M. from $n=6$. * $P<0.05$ significantly different from control, one-way ANOVA followed by Bonferroni multiple comparison t-test.

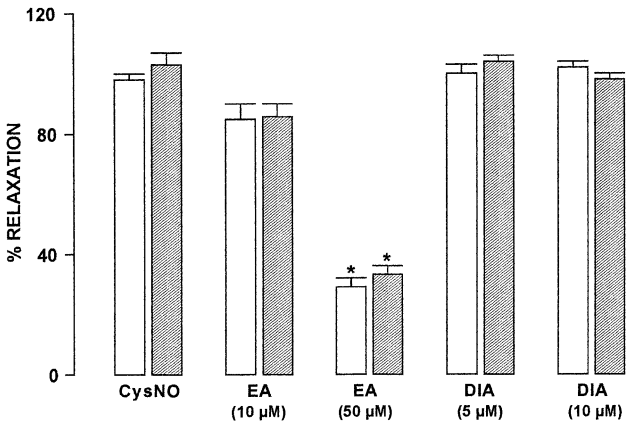

Fig. 4. Effects of ethacrynic acid (EA; 10 and 50 μM) and diamide (DIA; 5 and 10 μM) on the relaxations due to 0.05 μM (open bar) and 0.1 μM (hatched bar) *S*-nitrosocysteine (CysNO) in the longitudinal strips of the mouse gastric fundus. Values are means ± S.E.M. from $n=6$. * $P<0.05$ significantly different from control, one-way ANOVA followed by Bonferroni multiple comparison t-test.

Furthermore, diamide, a glutathione oxidizer that depletes intracellular glutathione, in a concentration of 5 μM which had no effect on the basal tone, significantly reduced the photorelaxation ($n=6$, Figs. 1D and 6). However, the higher concentrations of diamide (10 and 50 μM) did not further inhibit the photorelaxation (data not shown), and the diamide-induced inhibition was less than that by ethacrynic acid. The inhibitory effect of diamide (5 μM) on the photorelaxation was prevented by addition of glutathione (10 μM; $n=6$, Figs. 1E and 6). In contrast to photorelaxation, diamide (5 and 10 μM) failed to reduce the relaxations induced by exogenous NO and isoproterenol ($n=6$, Fig. 6). However, 50 μM diamide inhibited the relaxation in response to exogenous NO ($n=6$, data not shown); therefore, the 50-μM concentration of diamide was not used in

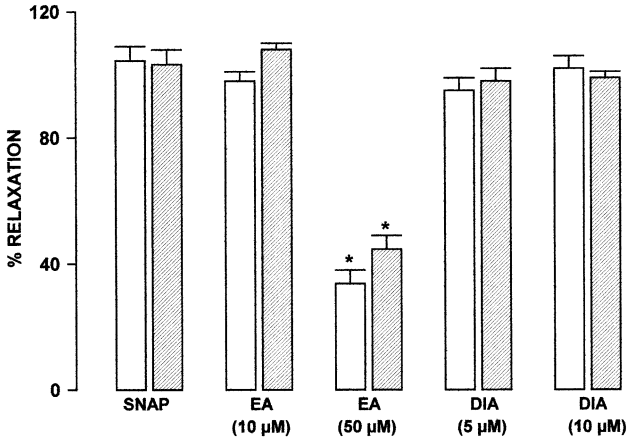

Fig. 5. Effects of ethacrynic acid (EA; 10 and 50 μM) and diamide (DIA; 5 and 10 μM) on the relaxations due to 0.5 μM (open bar) and 1 μM (hatched bar) *S*-nitroso-*N*-acetyl-penicillamine (SNAP) in the longitudinal strips of the mouse gastric fundus. Values are means ± S.E.M. from $n=6$. * $P<0.05$ significantly different from control, one-way ANOVA followed by Bonferroni multiple comparison t-test.

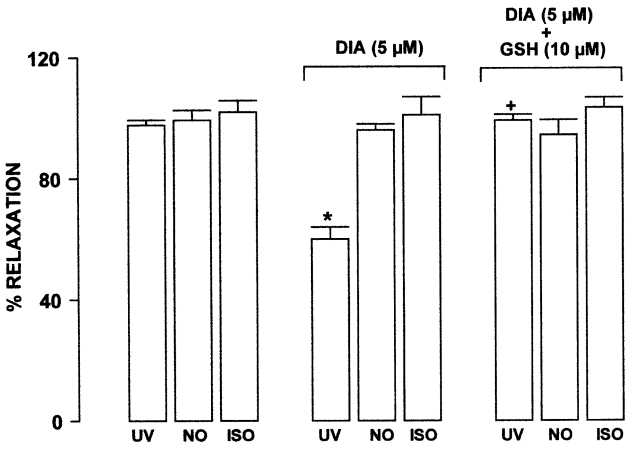

Fig. 6. Effects of diamide (DIA; 5 μM) alone and diamide plus glutathione (GSH; 10 μM) on the relaxations induced by UV irradiation (366 nm, 60 s), exogenous NO (10 μM) and isoproterenol (5 nM) in the longitudinal strips of the mouse gastric fundus. Values are means ± S.E.M. from $n=6$. * $P<0.05$ significantly different from control; $^{+}P<0.05$ significantly different from diamide (5 μM), one-way ANOVA followed by Bonferroni multiple comparison t-test.

experiments including *S*-nitrosothiol groups. Furthermore, diamide, in a concentration of 5 μM, significantly inhibited relaxations induced by *S*-nitrosoglutathione (0.1–1 μM), while 10 μM diamide abolished *S*-nitrosoglutathione-induced relaxation ($n=6$, Fig. 3). On the other hand, *S*-nitroso-L-cysteine- and *S*-nitroso-*N*-acetyl-penicillamine-induced relaxations were not inhibited by diamide (5 and 10 μM; $n=6$, Figs. 4 and 5).

4. Discussion

In the present study, the source of NO released by UV irradiation was investigated and our results have shown that *S*-nitrosoglutathione, at least in part, is the putative *S*-nitrosothiol that is converted to NO in response to UV irradiation in mouse gastric fundus strips.

Although the rabbit stomach muscle cells were found to be insensitive to UV light (Ehrreich and Furchgott, 1968), there appears to be a photosensitive material store in the mouse gastric fundus (Ögülener et al., 1996). This intrinsic photorelaxation was blocked by certain pharmacological substances that are known to inhibit NO-mediated relaxations, such as haemoglobin, hydroxocobalamin, $FeSO_4$ and methylene blue, providing evidence for NO as a photosensitive material store being responsible for the photorelaxation. However, N^G-methyl-L-arginine, a NO synthase inhibitor, and tetrodotoxin, a blocker of neuronal conductance, had no effect on these relaxations, ruling out the role of enzymatic synthesis of NO in photorelaxation. In the present study, the relaxation induced by UV light was shown to be inhibited by ODQ, a soluble guanylyl cyclase inhibitor, which was consistent with the finding of Lovren and Triggle (1998), and also corroborated our previous conclusion that the NO–cyclic GMP pathway takes part in photorelaxation. This finding is in contrast with the conclusion of Goud et al. (1996), who rejected the hypothesis that photorelaxation results from activation of the NO–cyclic GMP pathway. In support of our findings, Kubaszewski et al. (1994) showed directly the release of NO via UV light by using a porphyrinic sensor in vascular smooth muscle. Recently, results of several studies have suggested that *S*-nitrosothiols could be the tissue source of finite NO-yielding photosensitive materials in vascular smooth muscle (Megson et al., 1995; Lovren and Triggle, 1998). Also, it has been shown that NO can react with some intracellular and membrane-bound thiol groups to form nitrosothiols which can be photoactivable (Singh et al., 1996a,b; Megson et al., 2000). In the mouse gastric fundus, the photoactivated release of NO from tissue may be critically dependent on the availability of intracellular thiols such as glutathione and L-cysteine. We, therefore, investigated the effects of free thiols, glutathione and L-cysteine, on the relaxations in response to UV irradiation, exogenous NO, isoproterenol and *S*-nitrosothiols. Although glutathione did not affect responses to UV irradiation, exogenous NO and isoproterenol, *S*-nitrosoglutathione-induced relaxations were unexpectedly and significantly inhibited by this agent, but only at the highest concentration used. A possible explanation for this discrepancy may be that the yield of NO from *S*-nitrosoglutathione is decreased by higher glutathione concentrations, while the amounts of glutathione, which penetrate into the tissue, were not sufficient to react with endogenous putative *S*-nitrosoglutathione. Unusual results have been reported concerning the reactions of *S*-nitrosothiols with high concentrations of thiols, particularly the reaction of *S*-nitrosoglutathione with glutathione (Singh et al., 1996a,b; Wong et al., 1998; Dicks et al., 1998). As the added thiol concentration is increased, the yield of NO decreases, to be replaced by ammonia as the main nitrogen product, together with smaller amounts of nitrogen product. In contrast to glutathione, addition of L-cysteine inhibited photorelaxations and relaxations induced by exogenous NO but not by isoproterenol and *S*-nitroso-L-cysteine in the present study. The inhibitory action of L-cysteine does seem to be due to superoxide anion generation (Jia and Furchgott, 1993), because previous addition of Cu^{2+}/Zn^{2+} superoxide dismutase prevented its action. The differential effect of L-cysteine alone on the responses to UV light and *S*-nitroso-L-cysteine gives a hint about the localization of the NO store, that is, NO may be released from prejunctional neurons and then may pass the synaptic cleft where it may be scavenged by superoxide anions liberated from L-cysteine. The inhibitory effect of haemoglobin and hydroxocobalamin on photorelaxation, shown in our previous study (Ögülener et al., 1996), also supports the hypothesis mentioned above. Although the concentration of exogenously applied L-cysteine is increased in the presence of Cu^{2+}/Zn^{2+} superoxide dismutase, photorelaxation and NO-induced relaxation were not affected. The ineffectiveness of both glutathione and L-

cysteine on the responses to UV irradiation suggest that endogenous tissue thiol levels of the mouse gastric fundus might be high enough for photorelaxation.

In order to further investigate whether the source of NO in photorelaxation of gastric fundus is *S*-nitrosothiol, we studied the effects of a thiol-depleting agent, ethacrynic acid, a nonspecific thiol alkylator (Li et al., 1994) on the relaxations due to UV irradiation, exogenous NO and *S*-nitrosothiols. Treatment of gastric fundus with ethacrynic acid significantly inhibited relaxations due to UV irradiation and *S*-nitrosothiols. These results are in agreement with those of Lovren and Triggle (1998), who showed that ethacrynic acid abolished photorelaxation in rat thoracic aorta, implying the significance of the free thiol groups in photorelaxation. A similar conclusion was reached by Megson et al. (1995) for intact arteries, in which re-priming of the store could be prevented by ethacrynic acid, who postulated that the photosensitive store of NO could be an intracellular nitrosothiol. The effect of ethacrynic acid on photorelaxation may be dependent on the depletion of endogenous glutathione leading to a decrease of the formation, and thus of the pool, of *S*-nitrosoglutathione. As with photorelaxation, ethacrynic acid significantly inhibited the relaxations due to *S*-nitrosoglutathione, *S*-nitroso-L-cysteine and *S*-nitroso-*N*-acetyl-penicillamine in the present study. However, these substances do not have to be formed as they are added exogenously, therefore the thiol occupied by NO may be alkylated by ethacrynic acid, thereby interfering with the release of NO. In contrast, exogenous NO donor-induced responses were not attenuated by either thiol modulator, supporting the hypothesis of *S*-nitrosothiols, rather than NO, being the source of UV-induced relaxation. Also, the relaxations induced by isoproterenol, which activates adenylyl cyclase via β-adrenoceptors (Honeyman et al., 1977), were not inhibited by thiol-depleting agents, ruling out the nonspecific inhibitory action of thiol-depleting agents on smooth muscle cells.

Recently, Megson et al. (2000) reported that the photosensitive store in vascular smooth muscle is generated from intracellular GSH, which is converted to *S*-nitrosoglutathione by nitrosating species that are formed ultimately from endothelium-derived NO, and *S*-nitrosoglutathione is an important component of the photodegradable store. Also, the authors postulated that photorelaxation is due to, at least in part, to the photochemical release of NO from *S*-nitrosoglutathione in vascular smooth muscle. In the present study, to investigate whether *S*-nitrosoglutathione is involved in photorelaxation in gastric fundus, we used diamide, a glutathione oxidizer that drives a reversible oxidation of glutathione to glutathione disulphide and depletes intracellular glutathione (Murphy et al., 1991). Also, it was reported that diamide brought about a significant decrease in endothelial glutathione levels (Hecker et al., 1992). Diamide inhibited relaxations due to UV irradiation to a lesser extent than did ethacrynic acid and those to *S*-nitrosoglutathione, but not those to *S*-nitroso-*N*-acetyl-penicillamine, *S*-nitroso-L-cysteine, exogenous NO and isoproterenol, suggesting *S*-nitrosoglutathione but not *S*-nitroso-*N*-acetyl-penicillamine and *S*-nitroso-L-cysteine as being the source of NO in photorelaxation. The relatively slight inhibitory effect of diamide on UV relaxation when compared with that of ethacrynic acid might be due to the limited ability of this drug to penetrate the tissue or might result from different efficacy of each drug at the side of action. Also, a nonspecific action of ethacrynic acid on thiol groups other than glutathione might cause this difference. Thiol-depleting agents were less effective to attenuate photorelaxation of gastric fundal strips compared than was *S*-nitrosoglutathione. The incomplete inhibition of photorelaxation by thiol agents also suggests that an additional source of NO may be involved as photosensitive substance, such as intracellular nitrite, nitrosylated compounds and unknown nitro-containing substances that could be all sensitive to UV light. Also, it is possible that the light-induced decomposition of *S*-nitrosothiols may occur intracellularly and be less susceptible to inhibition by thiol-reducing agents than *S*-nitrosoglutathione, which was directly added into the bathing fluid that contained thiol-depleting agents.

There is further evidence supporting the proposal that the source of NO is *S*-nitrosoglutathione from the experiments using thiols on the inhibitory effects of thiol depletors on photorelaxation. The inhibitory effects of ethacrynic acid and diamide on photorelaxation were prevented by pretreating the tissues with glutathione. The fact that glutathione completely reversed the inhibitory effects of thiol depletors could be interpreted as prevention of the interaction of ethacrynic acid and diamide with thiol, suggesting that the thiols might play a direct role in the photorelaxation of mouse gastric fundus. In contrast to glutathione, L-cysteine did not prevent the inhibitory effect of ethacrynic acid on photorelaxation, implying that the photosensitive store is generated mainly from intracellular glutathione, and that endogenous *S*-nitrosoglutathione contributes to the molecular store of NO in gastric fundus. The decomposition of *S*-nitrosothiols by photolysis is well understood. UV light causes a homolytic cleavage of the sulfur–nitrogen bond, resulting in the release of NO and a thiyl radical (Singh et al., 1995). Also, it has been shown that NO can be readily liberated from *S*-nitrosoglutathione by visible radiation (340 or 545 nm) (Sexton et al., 1994). Our results obtained in the present experiments are consistent with those of Megson et al. (2000) and we suggest that the photorelaxation is mediated, at least in part, by NO released by the light-induced decomposition of *S*-nitrosoglutathione in nonvascular smooth muscle.

In conclusion, this study provided evidence that relaxation of mouse gastric smooth muscle by UV light is, at least in part, due to NO release into solution by catalysis of the decomposition of an endogenous "store" of *S*-nitrosothiols such as *S*-nitrosoglutathione. The physiological significance of such a photodepletable store needs to be clarified, but there could be several mechanisms that would serve as NO

generators. L-Arginine: NO synthetic pathway is one of the best known mechanisms that operates on neuronal demand, and adaptive relaxation of the fundus region during food intake is mainly mediated by nitrergic nerves in mouse gastric fundus (Ny and Andersson, 1998; Ergün and Ögülener, 2001). Since replenishment of the photosensitive store by long-term electrical stimulation in mouse gastric fundus was shown by Büyükavşar et al. (1999), a role for *S*-nitrosothiols as a complementary pathway in maintaining this adaptive relaxation might be considered, or alternatively, *S*-nitrosothiols might play a role as a back-up system to modulate the basal tone of the gastrointestinal tract via the continuous release of NO.

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