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# A possible role of *S*-nitrosothiols at the nitrergic relaxations in the mouse corpus cavernosum

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**Abstract**

Relaxations induced by electrical field stimulation and acetylcholine were compared with those induced by acidified sodium nitrite, sodium nitroprusside, *S*-nitrosoglutathione and *S*-nitroso-*N*-acetyl-D,L-penicillamine in the mouse corpus cavernosum precontracted with phenylephrine. $N^G$-nitro-L-arginine inhibited electrical field stimulation- or acetylcholine-induced relaxation, but was ineffective on relaxations caused by the other stimuli. Hydroquinone and pyrogallol had no inhibitory action on the relaxations caused by any stimulus except acidified sodium nitrite. Incubation of the tissue with diethyldithiocarbamic acid significantly inhibited the relaxations induced by all stimuli except papaverine. In the tissues pre-treated with diethyldithiocarbamic acid, superoxide dismutase, hydroquinone and pyrogallol failed to yield restore or further inhibit the relaxations in response to electrical field stimulation or acetylcholine. LY 83583 (6-anilino-5,8-quinolinedione) and hydroxocobalamin clearly inhibited the relaxant responses to electrical field stimulation, acetylcholine, *S*-nitrosoglutathione and acidified sodium nitrite whereas there was significant enhancement of the relaxation produced by *S*-nitroso-*N*-acetyl-D,L-penicillamine. These findings suggest that the relaxant factor released from non-adrenergic non-cholinergic nerves or endothelial cells in mouse cavernosal tissue may be a superoxide anion-resistant nitric oxide-containing molecule and that *S*-nitrosoglutathione rather than *S*-nitroso-*N*-acetyl-D,L-penicillamine could be a suitable candidate for this. 

*Keywords:* Corpus cavernosum; Mouse; *S*-nitrosothiol; NANC (non-adrenergic non-cholinergic) nerve; Nitric oxide (NO)

## 1. Introduction

The modulator role of nitric oxide (NO) in penile erection has recently been the subject of extensive investigation. NO is synthesized by both the endothelium lining the lacunar spaces and the autonomic dilator nerves of the cavernous tissue, and mediates trabecular smooth muscle relaxation via accumulation of cGMP (Saenz de Tejada, 1992). The role of NO in non-adrenergic non-cholinergic (NANC) neurotransmission is now well established, but the exact chemical structure of the nitrergic neurotransmitter remains to be identified. It was thought that NO may not be transported as the free form, but as a nitrosothiol molecule from the synthesizing cell to the target cell in some tissues, based on observations that hydroquinone and pyrogallol, superoxide anion generators or free radical scavengers (Mittal and Murad, 1977; Griffith et al., 1984; Gillespie and Sheng, 1990) have no effect on the relaxation induced by electrical field stimulation of NANC nerves in the mouse anococcygeus (Hobbs et al., 1991; Gibson et al., 1992) and bovine retractor penis (Gillespie and Sheng, 1990; Liu et al., 1994) muscles. However, results of subsequent studies performed on the same tissues and on rat anococcygeus muscle in the presence of diethyldithiocarbamate, an inhibitor cytosolic or $Zn^{2+}$–$Cu^{2+}$ form of superoxide dismutase (Kelner et al., 1989), revealed that the failure of hydroquinone and pyrogallol to inhibit the relaxation elicited by electrical field stimulation may have resulted from high superoxide dismutase activity of these tissues (Liu and Szurszewski, 1994; Martin et al., 1994; Lilley and Gibson, 1995). On the other hand, effects of some *S*-nitrosothiols have been investigated in the bovine retractor penis and rat gastric fundus regarding the suggestion that NO is not transported freely from synthe-

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sizing cell to the target cell (Barbier and Lefebvre, 1994; Liu et al., 1994). Contradictory findings have been obtained from these studies, regarding the suitability of *S*-nitrosoglutathione as possible nitrergic neurotransmitter.

In our previous study on the mouse corpus cavernosum, results obtained in the presence of hydroquinone favoured the view that the mediator released from NANC nerves (tetrodotoxin-sensitive) and endothelial cells may not be free NO, but a superoxide-resistant, NO-generating molecule (Göçmen et al., 1997). However, the tissue should have been retested with superoxide anion generating agents after diethyldithiocarbamic acid preincubation to find the true character of the mediator. The aim of the present study was to investigate whether there is an actual contribution of free NO to the relaxation induced by electrical field stimulation or acetylcholine and whether there are dissimilarities between the nature of the relaxation evoked by electrical field stimulation, acetylcholine, acidified sodium nitrite, sodium nitroprusside, *S*-nitrosoglutathione and *S*-nitroso-*N*-acetyl-D,L-penicillamine in the mouse cor-

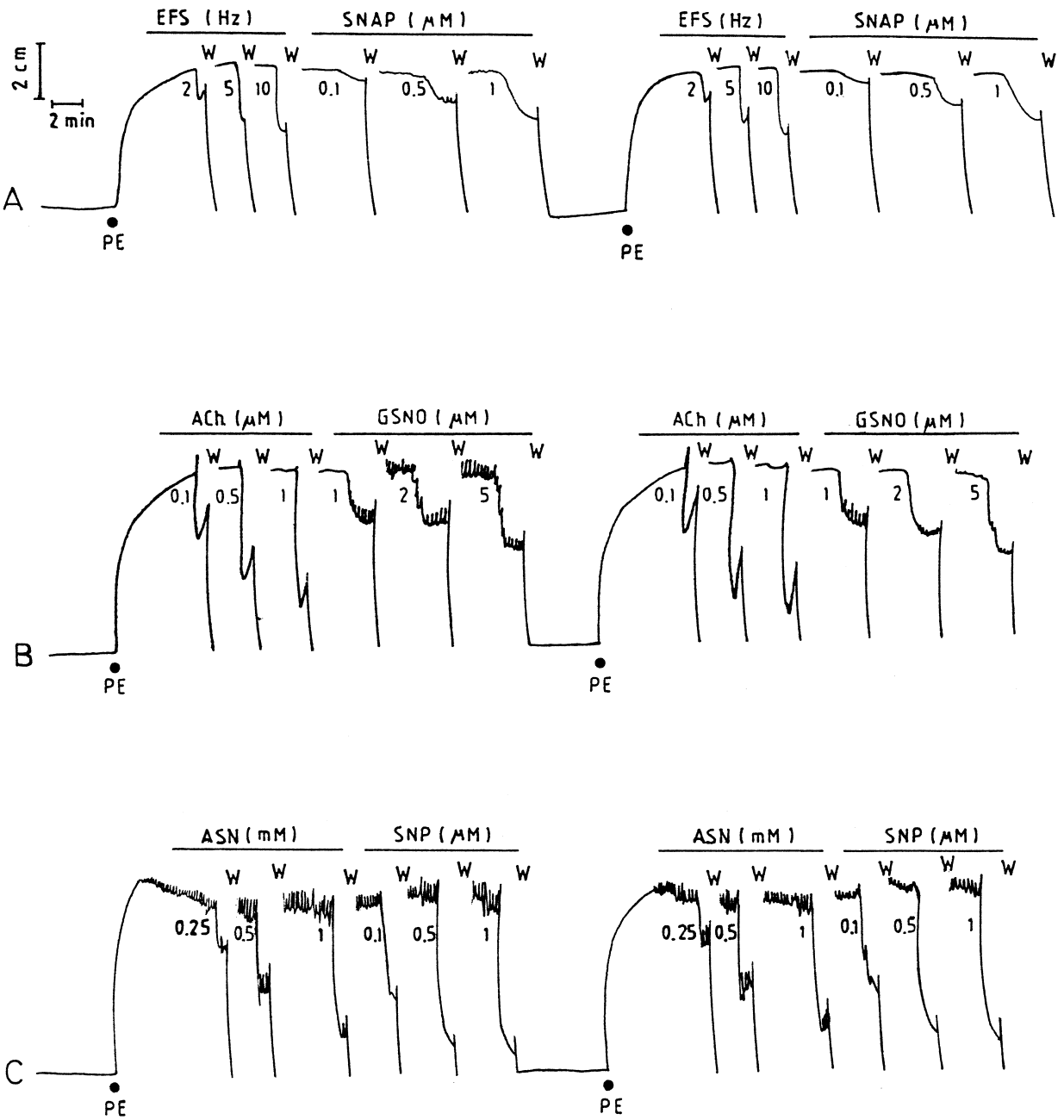


Fig. 1. Representative tracings showing relaxant effects of electrical field stimulation (EFS) or *S*-nitroso-*N*-acetyl-D,L-penicillamine (SNAP) (A), acetylcholine (ACh) or *S*-nitrosoglutathione (GSNO); (B) and acidified sodium nitrite (ASN) or sodium nitroprusside (SNP); (C) in the mouse corpus cavernosum precontracted with 5 μM phenylephrine (PE). Following each application of EFS or chemicals, recording was stopped and the tissue was washed (w) and after 15 min PE was added to the bathing medium and recording was restarted when the tissue tone reached to near previous level.

pus cavernosum by using several agents affecting nitrergic transmission.

## 2. Materials and methods

### 2.1. Organ bath experiments

Male albino mice weighing 30–40 g were killed by cervical dislocation. Penises were removed and then placed in a Petri dish containing Krebs solution (composition mM: NaCl, 119; KCl, 4.6; $CaCl_2$, 1.5; $MgCl_2$, 1.2; $NaHCO_3$, 15; $NaHPO_4$, 1.2; glucose, 11). The glans penis and urethra were excised. Two corpus cavernosums were separated by cutting the fibrous septum between them. Each corpus cavernosum was carefully dissected from the adherent tissues, keeping the tunica albuginea intact. The preparations were mounted under 0.2 g tension in 5-ml organ baths maintained at 37°C and containing Krebs solution aerated with 95% $O_2$ and 5% $CO_2$. In experiments in which electrical field stimulation was used, atropine (0.2 μM) and guanethidine (1 μM) were always present in the bathing medium to obtain NANC conditions. The tissues were allowed to equilibrate for 1 h during which they were washed with fresh Krebs solution at 15 min intervals. The responses were recorded with isotonic transducers (Ugo Basile, 7006) on polygraph paper (Ugo Basile, Gemini 7070). After the equilibration period the tissues were treated with 5 μM phenylephrine. This resulted in an active tone that reached a stable level within 5 min; at the end of this period, electrical field stimulation delivered as square waves (2, 5 and 10 Hz; 10 V, 2 ms) by a Grass S88 stimulator via two parallel platinum electrodes embedded in Perspex was applied to the tissue for 30 s at 15 min intervals. In some experiments acetylcholine (0.1, 0.5 and 1 μM), acidified sodium nitrite (0.25, 0.5 and 1 mM) sodium nitroprusside (0.1, 0.5 and 1 μM), *S*-nitrosoglutathione (1, 2 and 5 μM) or *S*-nitroso-*N*-acetyl-D,L-penicillamine (0.1, 0.5 and 1 μM) was added to the bathing medium after the tissue reached steady state of the contraction produced by phenylephrine. Exposure time of the tissue was 2 min for *S*-nitrosoglutathione and *S*-nitroso-*N*-acetyl-D,L-penicillamine, 1 min for other chemicals. Following each application of electrical field stimulation or chemicals, recording was stopped and the tissue was washed with fresh Krebs solution and thus relaxed back to the baseline. After 15 min, 5 μM phenylephrine was added into the bath and recording was restarted when the tissue tone reached to near the previous tone. The procedure was repeated by using the next concentration of the chemicals or frequency of electrical stimulation until all concentrations or frequencies had been applied. Thus the first series of responses was obtained. After the tissue was left to rest for 30 min, the second series of responses was recorded as control in the same manner. In some trials, after the first three control responses were recorded, the preparation was replaced into the medium with $N^G$-nitro-L-arginine (10 μM), hydroquinone (100 μM), pyrogallol (50 μM), LY 83583 (5 or 10 μM) or hydroxocobalamin (100 or 200 μM) and the second series of relaxation due to chemicals or electrical field stimulation was examined.

In another series of experiments, after the first three responses to electrical field stimulation (2–10 Hz), acetylcholine (0.1–1 μM), acidified sodium nitrite (0.25–1 mM) sodium nitroprusside (0.1–1 μM), *S*-nitrosoglutathione (1–5 μM) or *S*-nitroso-*N*-acetyl-D,L-penicillamine (0.1–1 μM) or papaverine (10 μM) had been recorded, the tissue was incubated with diethyldithiocarbamic acid (8 mM) for 30 min and washed with fresh Krebs solution. Responses of the preparation to various stimuli were determined 15 min later. The preliminary experiments performed with diethyldithiocarbamic acid were done with a previously described method (Martin et al., 1994). In these experiments, the action of the agent on the responses to electrical field stimulation and acetylcholine was examined at different concentrations (1, 2, 4 and 8 mM) by testing several incubation periods (30, 60 and 120 min). The most suitable

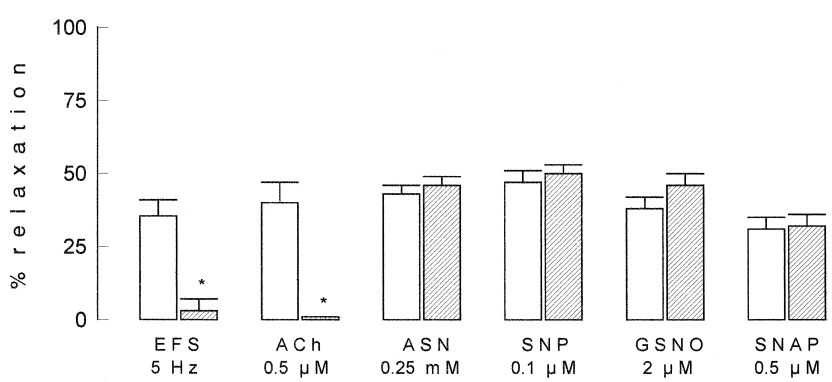


Fig. 2. The relaxant responses to electrical field stimulation (EFS), acetylcholine (ACh), acidified sodium nitrite (ASN), sodium nitroprusside (SNP), *S*-nitrosoglutathione (GSNO) and *S*-nitroso-*N*-acetyl-D,L-penicillamine (SNAP) in the absence (open bars) and presence (hatched bars) of 10 μM $N^G$-nitro-L-arginine in the mouse corpus cavernosum precontracted with 5 μM phenylephrine. Each column represents the mean relaxant response expressed as percentage peak reduction by phenylephrine. $* P < 0.05$ ($n = 6–12$).

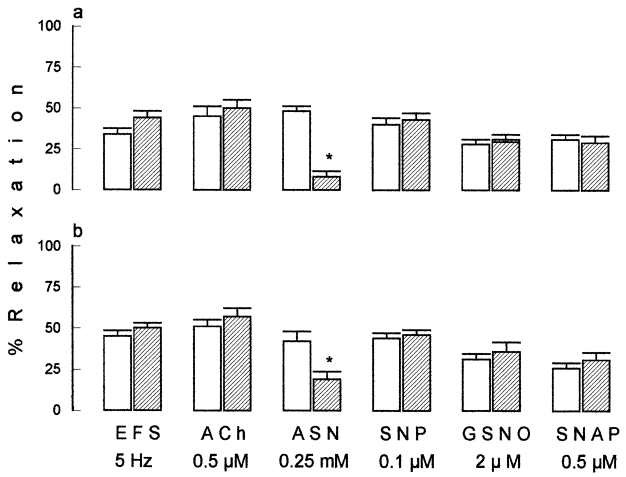


Fig. 3. The relaxant responses to electrical field stimulation (EFS), acetylcholine (ACh), acidified sodium nitrite (ASN), sodium nitroprusside (SNP), *S*-nitrosoglutathione (GSNO) and *S*-nitroso-*N*-acetyl-D,L-penicillamine (SNAP) in the absence (open bars) and presence (hatched bars) of 100 μM hydroquinone (a) and 50 μM pyrogallol (b) in the mouse corpus cavernosum precontracted with 5 μM phenylephrine. Each column represents the mean relaxant response expressed as percentage peak reduction of phenylephrine contraction. $* P < 0.05$ ($n = 6–14$).

concentration and incubation time with diethyldithicarbamic acid for the tissue used were 8 mM and 30 min, respectively. Under these conditions, the contraction due to phenylephrine was more consistent and there was a significant reduction in electrical field stimulation- and acetylcholine-induced relaxations. One group of experiments served to evaluate responses of the tissue preincubated with diethyldithiocarbamic acid (8 mM) + $CuCl_2$ (8 mM) to all stimuli. In a subgroup of experiments, the tissue was replaced into the medium with hydroquinone (100 μM) or pyrogallol (50 μM) after washing out diethyldithiocarbamic acid; 10 min later, the relaxant responses were examined. In another experimental subgroup, after the first three responses to electrical field stimulation (5 Hz) were recorded (without washing), the tissue was incubated with diethyldithiocarbamic acid (8 mM) for 30 min. Diethyldithiocarbamic acid was washed out and six successive electrical stimulation were applied without washing of the tissue. In some trials, superoxide dismutase (250 U/ml), pyrogallol or hydroquinone was added into the bath after three of six successive electrical stimulation were delivered to examine the immediate action of the drugs. The same protocol was also performed for acetylcholine (0.5 μM) through after each application acetylcholine was washed out with fresh Krebs solution.

### 2.2. Drugs and solutions

Stock solutions of atropine sulphate, phenylephrine, acetylcholine, sodium nitroprusside, papaverine, hydroxocobalamin, cupric chloride ($CuCl_2$) and diethyldithiocarbamate were dissolved in distilled water. *S*-nitrosoglutathione and *S*-nitroso-*N*-acetyl-D,L-penicillamine solutions were prepared immediately before use and kept at −4°C. Guanethidine was dissolved in dimethyl sulfoxide (0.1% v/v). LY 83583 (6-anilino-5,8-quinolinedione) was made up as a 20 mM stock in ethanol. $N^G$-nitro-L-arginine, hydroquinone and pyrogallol were prepared daily in Krebs solution. Nitric oxide was present as acidified solution (pH 2) of $NaNO_2$ and stored at −4°C. Superoxide dismutase ($Cu^{2+}/Zn^{2+}$-containing enzyme from bovine erythrocytes) was dissolved in saline (0.9%). All the drugs were obtained from Sigma, except $NaNO_2$ (Merck), LY 83583 (RBI) and *S*-nitroso-*N*-acetyl-D,L-penicillamine (RBI).

### 2.3. Statistical considerations

Relaxations were calculated as percentage peak reductions of phenylephrine contraction. The mean values (±S.E.) for the untreated series and for the treated series were calculated separately for each experimental group. All data were evaluated in a One-way analysis of variance

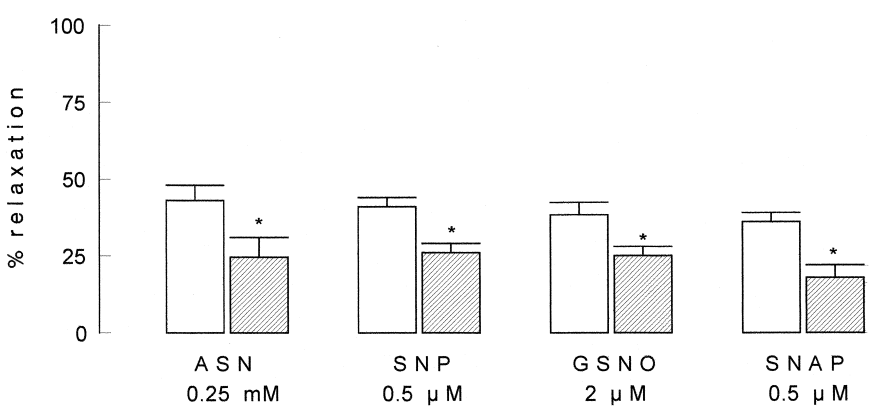


Fig. 4. Relaxations induced by acidified sodium nitrite (ASN), sodium nitroprusside (SNP), *S*-nitrosoglutathione (GSNO) and *S*-nitroso-*N*-acetyl-D,L-penicillamine (SNAP) in the tissue untreated (open bars) or pre-treated with 8 mM diethyldithiocarbamic acid (hatched bars) in the mouse corpus cavernosum precontracted with 5 μM phenylephrine. Each column represents the mean relaxant response expressed as percentage peak reduction of phenylephrine contraction. $* P < 0.05$ ($n = 6–12$).

Table 1
Ineffectiveness of hydroquinone (HQ) or pyrogallol on the responses to electrical field stimulation (EFS) or acetylcholine (ACh) in the mouse corpus cavernosum pretreated with diethyldithiocarbamic acid (DETCA)

| | EFS (5 Hz) | ACh (0.5 μM) |
|---|---|---|
| DETCA (8 mM) | 28 ± 6.2 ($n$ = 10) | 26 ± 5.0 ($n$ = 10) |
| DETCA (8 mM) + HQ (100 μM) | 31 ± 7.6 ($n$ = 8) | 24 ± 4.1 ($n$ = 8) |
| DETCA (8 mM) + pyrogallol (50 μM) | 29 ± 5.1 ($n$ = 12) | 27 ± 4.6 ($n$ = 10) |

Values are presented as percent of control inhibition (mean ± S.E.).

(ANOVA), using a computer program, SPSS. *P*-values of less than 0.05 were considered to be significant.

## 3. Results

### 3.1. Relaxant effects of electrical field stimulation, acetylcholine, acidified sodium nitrite, sodium nitroprusside and S-nitrosothiols in the mouse corpus cavernosum

Electrical field stimulation ($n$ = 10), acetylcholine ($n$ = 10), acidified sodium nitrite ($n$ = 10) and sodium nitroprusside ($n$ = 10) all relaxed the mouse corpus cavernosum in a frequency- or concentration-dependent manner. The two *S*-nitrosothiols: *S*-nitrosoglutathione and *S*-nitroso-*N*-acetyl-D,L-penicillamine induced reproducible, concentration-dependent relaxations that were relatively slow to develop when compared to electrically- or acetylcholine-induced relaxation (Fig. 1).

### 3.2. Effects of $N^G$-nitro-L-arginine, hydroquinone and pyrogallol

$N^G$-nitro-L-arginine (10 μM) inhibited both electrically and acetylcholine-induced relaxation, but did not affect the relaxation produced by acidified sodium nitrite, sodium nitroprusside and *S*-nitrosothiols (Fig. 2). Hydroquinone (100 μM) and pyrogallol (50 μM) did not inhibit the relaxations induced by electrical field stimulation, acetylcholine, sodium nitroprusside or *S*-nitrosothiols while they reduced the relaxant effect of acidified sodium nitrite (Fig. 3).

### 3.3. Effect of diethyldithiocarbamic acid incubation

Diethyldithiocarbamic acid (8 mM, for 30 min, followed by washout) caused a significant inhibition, which was prevented by $CuCl_2$ (8 mM), of the relaxations induced by electrical stimulation and all relaxant substances (Fig. 4 and Table 1) except papaverine (10 μM, data not shown) elicited relaxation. In the tissue preincubated with diethyldithiocarbamic acid, hydroquinone (100 μM) or pyrogallol (50 μM) applied at the end of a 10-min incubation or immediately before electrical stimulation or acetylcholine application, failed to yield any further inhibition of the relaxation induced by electrical stimulation or acetylcholine ($n$ = 8–10; Fig. 5; Table 1). Addition of exogenous superoxide dismutase (250 U/ml) did not cause any restoration of the relaxation induced by electrical stimulation at the frequency of 5 Hz (as percent inhibition of control: diethyldithiocarbamic acid 22.16 ± 2.11 vs. diethyldithiocarbamic acid + superoxide dismutase 22.9 ± 2.08, $n$ = 6) and 0.5 μM acetylcholine (as percent inhibition of control: diethyldithiocarbamic acid 22.8 ± 4.15 versus diethyldithiocarbamic acid + superoxide dismutase 20.5 ± 4.5, $n$ = 7) in diethyldithiocarbamic acid pretreated tissue.

### 3.4. Effects of LY 83583 and hydroxocobalamin

LY 83583 (5 and 10 μM) caused a significant inhibition of the relaxant responses to electrical stimulation, acetylcholine, acidified sodium nitrite and *S*-nitrosoglutathione whereas it left sodium nitroprusside action unaffected and significantly enhanced the response to *S*-nitroso-*N*-acetyl-D,L-penicillamine (Fig. 6a). The inhibitory effect of LY 83583 on acidified sodium nitrite-induced relaxation was much greater than the effects of other substances (88 ± 7.9% for acidified sodium nitrite vs. 51

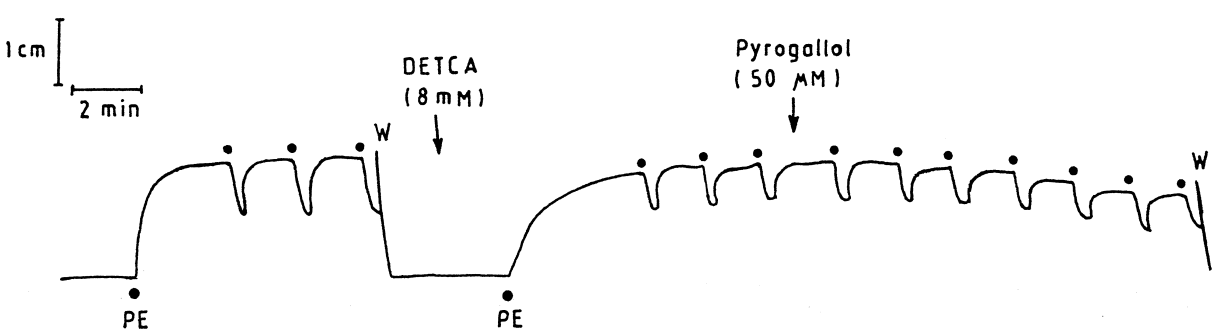


Fig. 5. Representative tracing showing ineffectiveness of pyrogallol (50 μM) on electrical field stimulation (EFS; 5 Hz)-induced relaxation in the tissue pretreated with 8 mM diethyldithiocarbamic acid (DETCA). (w) Washout, (●) indicates EFS application, (PE) phenylephrine (5 μM).

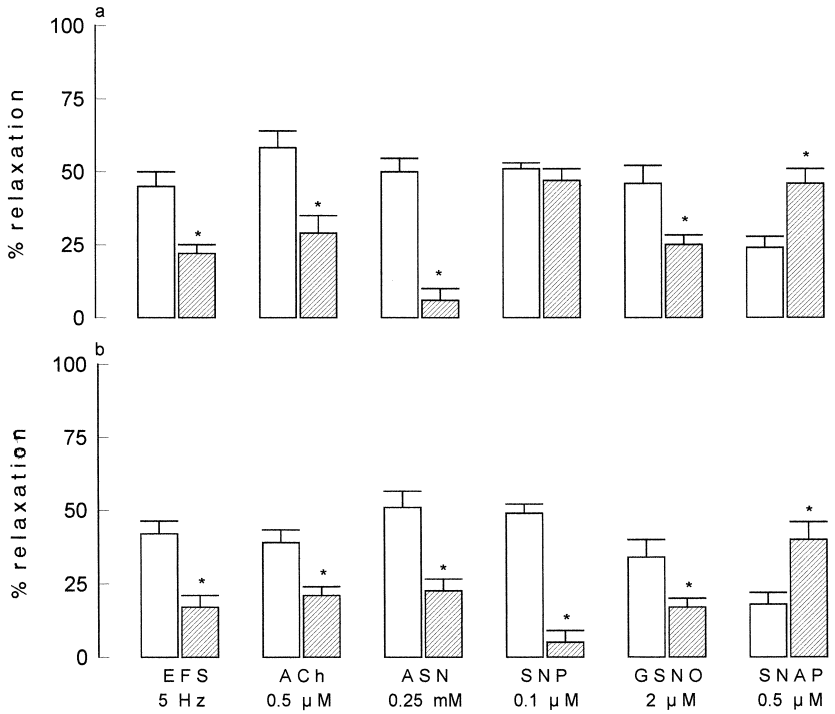


Fig. 6. The relaxant responses to electrical field stimulation (EFS), acetylcholine (ACh), acidified sodium nitrite (ASN), sodium nitroprusside (SNP), *S*-nitrosoglutathione (GSNO) and *S*-nitroso-*N*-acetyl-D,L-penicillamine (SNAP) in the absence (open bars) and presence (hatched bars) of 10 μM LY 83583 (a) and 100 μM hydroxocobalamin (b) in the mouse corpus cavernosum precontracted with 5 μM phenylephrine. Each column represents the mean relaxant response expressed as percentage peak reduction of phenylephrine contraction. $* P < 0.05$ ($n = 8–12$).

$\pm 6$, $49 \pm 10.2$, and $46.3 \pm 7\%$ for electrical stimulation, acetylcholine and *S*-nitrosoglutathione, respectively). The relaxations induced by electrical field stimulation, acetylcholine, acidified sodium nitrite, sodium nitroprusside or *S*-nitrosoglutathione were significantly reduced by hydroxocobalamin (100 and 200 μM) (Fig. 6b). In contrast, hydroxocobalamin caused a significant potentiation of the relaxant responses to *S*-nitroso-*N*-acetyl-D,L-penicillamine.

## 4. Discussion

The results of the present study suggest that the relaxant mediator released from NANC nerves and endothelial cells in the mouse corpus cavernosum may have an NO containing superoxide anion resistant molecule displaying *S*-nitrosoglutathione-like character but not free NO.

Electrical field stimulation, acetylcholine, acidified sodium nitrite, sodium nitroprusside, *S*-nitrosoglutathione and *S*-nitroso-*N*-acetyl-D,L-penicillamine produced relaxation in a frequency- or concentration-dependent manner. Sodium nitroprusside, *S*-nitrosoglutathione and *S*-nitroso-*N*-acetyl-D,L-penicillamine caused a relaxation resembling effects of electrical field stimulation or acetylcholine, but their actions were relatively slow to develop. The ineffectiveness of $N^G$-nitro-L-arginine on responses of the tissue to all stimuli used, except electrical stimulation and acetylcholine, suggests that acidified sodium nitrite, sodium nitroprusside and two *S*-nitrosothiols have a direct action on the mouse corpus cavernosum. These findings are consistent with results of previous studies on vascular and non-vascular tissues (Henry et al., 1989; Gibson et al., 1992; Jansen et al., 1992; Barbier and Lefebvre, 1994; Boeckxstaens et al., 1994; De Man et al., 1995).

Hydroxocobalamin, an NO binding agent (Rajanayagam et al., 1993), enhanced *S*-nitroso-*N*-acetyl-D,L-penicillamine-induced relaxation markedly, while it caused a significant reduction in responses to other stimuli used, indicating that the mediator released by electrical stimulation from NANC nerves and by acetylcholine from endothelial cells may not be identical to *S*-nitroso-*N*-acetyl-D,L-penicillamine. On the other hand, the inhibitory actions of hydroxocobalamin on the relaxations elicited by electrical stimulation, acetylcholine and acidified sodium nitrite were similar, confirming the result obtained from a previous study on the same tissue (Göçmen et al., 1997). Although, this observation does not favor the suggestion that hydroxocobalamin is a useful tool to discriminating between exogenous NO and an endogenous nitrergic neurotransmitter (Rajanayagam et al., 1993; Lefebvre, 1996), the result may depend on the concentration of hydroxocobalamin used and/or on the tissue under study, and it does not exclude usefulness of hydroxocobalamin for discriminating between NO and the nitrergic neurotransmitter in other tissues (La et al., 1997).

An interesting finding of the present study was that an extra- and intracellular superoxide anion generating agent, LY 83583 (Mülsch et al., 1988), reduced the responses to electrical stimulation, acetylcholine, acidified sodium nitrite and *S*-nitrosoglutathione, but not those to sodium nitroprusside and *S*-nitroso-*N*-acetyl-D,L-penicillamine. This finding suggests that the relaxant mediator released from NANC nerves or endothelial cells may not have an sodium nitroprusside or *S*-nitroso-*N*-acetyl-D,L-penicillamine-like molecule. Since it has been shown that LY 83583 caused the generation of intracellular superoxide anions, leading to inhibition of the relaxation induced by activation of the NO/cGMP pathway (Cherry et al., 1990), it is difficult to explain why the agent did not reduce the action of sodium nitroprusside or *S*-nitroso-*N*-acetyl-D,L-penicillamine known as NO donors exerting their effects via cGMP production (Ignarro et al., 1981; Rapoport and Murad, 1983; Barbier and Lefebvre, 1994). However, in a previous study on rabbit thoracic aorta, an increase in the sodium nitroprusside-induced cGMP formation was found when the tissue was pretreated with LY 83583 (Gupta et al., 1994). Although the mechanical response was not monitored in the latter study, this finding suggests that LY 83583 may increase the cGMP production induced by an NO containing agent in some tissues. The finding in the present study that the inhibitory effect of LY 83583 on acidified sodium nitrite-induced relaxation was much greater than the effects of other substances may be attributed to the additional contribution of extracellular superoxide anion generation to the inhibition. The reduction in the response to electrical stimulation and acetylcholine was similar to that by *S*-nitrosoglutathione which generates NO near or at the membrane. This finding suggests that the endogenous mediator responsible for the action of electrical stimulation and acetylcholine may be resistant to extracellular superoxide anions.

Diethyldithiocarbamic acid, an irreversible inhibitor of $Cu^{2+}$–$Zn^{2+}$ superoxide dismutase, found in either cytosol or the extracellular milieu, did inhibit relaxations produced by electrical field stimulation and acetylcholine. The prevention of this inhibition by $CuCl_2$ suggests that diethyldithiocarbamic acid is a specific copper chelator hence, it may affect superoxide dismutase activity by binding $Cu^{2+}$ in the molecule (Liu et al., 1997). However, the inhibitory action of diethyldithiocarbamic acid was not reversed by superoxide dismutase (250 U/ml) which cannot enter cells (Salvemini and Botting, 1993; Martin et al., 1994). Therefore, it is possible to say that the diethyldithiocarbamic acid-induced decrease in the relaxation may not have resulted from the extracellular superoxide dismutase inhibition. In addition, the finding supporting this view in the present study was that diethyldithiocarbamic acid inhibited the response to exogeneously applied acidified sodium nitrite, as well as actions of sodium nitroprusside and the two *S*-nitrosothiols which possess molecules resistant to extracellular superoxide anions. Some evidence suggesting the presence of an intracellular mechanism activated by diethyldithiocarbamic acid has been obtained from the studies on calf coronary artery, rabbit thoracic aorta and rat gastric fundus (Mügge et al., 1991; Omar et al., 1991; Lefebvre, 1996). The inhibitory action of diethyldithiocarbamic acid seems to be specific for the L-arginine/NO/cGMP pathway, since it did not reduce the relaxation induced by papaverine.

On the other hand, hydroquinone and pyrogallol, scavenger and/or superoxide generating agents which cannot penetrate into cells (Ignarro et al., 1988), did not affect relaxations except acidified sodium nitrite-evoked responses. Moreover, after incubation of the tissue with diethyldithiocarbamic acid, the substances were still ineffective on the relaxation induced by electrical stimulation or acetylcholine. Similar results were obtained from the study on the rat gastric fundus (Lefebvre, 1996). These observations suggest that the inhibition of the extracellular superoxide dismutase may not be sufficient to reduce the prevention of the mediator from superoxide anions in the extracellular fluid. In the other words, the mediator released from nitrergic nerves or endothelial cells must have been preserved or somehow unaffected by the inhibition of the extracellular superoxide dismutase, indicating that the relaxant factor may not be free NO and/or that there may be a powerful mechanism including the presence of endogenous antioxidant substances, such as ascorbate and urate, for NO in the extracellular space (Lilley and Gibson, 1997). However, findings from previous studies on tissues such as the bovine retractor penis, rat and mouse anococcygeus muscles, do not support our results (Liu and Szurszewski, 1994; Martin et al., 1994; Lilley and Gibson, 1995).

In conclusion, the relaxant mediator released from NANC nerves and endothelial cells in mouse corpus cavernosum may not be free NO but an NO containing molecule, since the differential effects of hydroquinone and pyrogallol on acidified sodium nitrite-induced and electrical field stimulation or acetylcholine-mediated relaxations were still observed after incubation of the tissue with diethyldithiocarbamic acid. The endogenous relaxant mediator may have a *S*-nitrosoglutathione-like structure because its behavior was similar to that of *S*-nitrosoglutathione in the presence of several pharmacological agents. On the other hand, it is well known that free NO has been suggested as the nitrergic neurotransmitter based on a number of studies on various tissues including mouse anococcygeus muscle (Lilley and Gibson, 1995). However, there are discrepant findings in the studies on the same tissue (De Man et al., 1996; Lefebvre, 1996). These contradictory results could be a consequence of differences in experimental conditions. Although the mechanism responsible for the transfer of NO to the NO-binding molecule remains to be identified, our findings may favour the hypothesis of a carrier molecule for NO (Gibson et al., 1995; Gibson and Lilley, 1997).

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