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Differentiation by hydroquinone of relaxations induced by exogenous and endogenous nitrates in non-vascular smooth muscle: role of superoxide anions

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1 The influence of hydroquinone on relaxations induced by nitric oxide (NO), nitrovasodilator drugs, and non-adrenergic, non-cholinergic (NANC) field stimulation has been investigated in three tissues in which endogenous nitrates have been implicated in the NANC response; the mechanism of action of hydroquinone was also studied.

2 In mouse anococcygeus, hydroquinone (10–100 μM) produced a concentration-dependent inhibition of relaxations induced by 15 μM NO. Hydroquinone, 100 μM, which reduced responses to NO by 85%, had no effect on relaxations induced by NANC field stimulation (10 Hz; 20 s trains), hydroxylamine (10 μM), sodium nitroprusside (1 μM) or sodium azide (20 μM).

3 In guinea-pig trachea, 100 μM hydroquinone reduced relaxations to 150 μM NO by 75%, but had no effect on those to NANC stimulation (10 Hz; 30 s trains) or sodium azide (5 μM).

4 In rat gastric fundus, 100 μM hydroquinone reduced relaxations to 1 μM NO by 85%, but had no effect on those to NANC stimulation (0.5 Hz; 15 s trains) or sodium azide (2 μM).

5 Superoxide dismutase (SOD; 50 u ml^{-1}) had no effect on relaxations of the mouse anococcygeus in response to 15 μM NO or 10 Hz NANC stimulation. Further, the inhibition of responses to NO by hydroquinone was unaffected in the presence of SOD.

6 Hydroquinone (10–100 μM) failed to generate superoxide anions, as detected by a chemiluminescent assay. However, 100 μM hydroquinone, like SOD (50 u ml^{-1}), produced almost complete inhibition of superoxide anion chemiluminescence induced by xanthine (500 μM): xanthine oxidase (0.07 u ml^{-1}).

7 It is concluded that, in our system, hydroquinone inhibits NO by acting as a free radical scavenger rather than by generating superoxide anions. The ability of hydroquinone to block relaxations to NO, but not NANC stimulation, may suggest that the endogenous nitrate substance released by these NANC nerves may not be free NO, but may be an NO-containing, or NO-generating, molecule.

Keywords: Guinea-pig trachea; hydroquinone; mouse anococcygeus; nitric oxide; nitrovasodilators; non-adrenergic, non-cholinergic; rat gastric fundus; superoxide anions; superoxide dismutase

Introduction

Certain non-adrenergic, non-cholinergic (NANC) relaxations may be mediated by the release of an endogenous nitrate neurotransmitter, generated via a system similar to that responsible for the production of endothelium-derived relaxing factor (EDRF). Thus, drugs known to inhibit EDRF synthesis, such as L-N^G-monomethyl-arginine (Moncada *et al.*, 1989) and L-N^G-nitro-arginine (L-NOARG; Moore *et al.*, 1990), reduce NANC relaxations in a variety of tissues, including the anococcygeus (Gillespie *et al.*, 1989; Ramagopal & Leighton, 1989; Gibson *et al.*, 1990; Hobbs & Gibson, 1990), trachea (Tucker *et al.*, 1990; Li & Rand, 1991), blood vessels (Toda & Okamura, 1990), gastrointestinal tract (Bult *et al.*, 1990; Boeckxstaens *et al.*, 1990; Li & Rand, 1990), and urogenital tract (Gillespie, 1990; Ignarro *et al.*, 1990a; Andersson *et al.*, 1991; Holmquist *et al.*, 1991). In addition, L-arginine:nitric oxide (NO) synthase, the enzyme involved in the generation of EDRF, has been detected by immunocytochemistry in nerve terminals in both the central and peripheral nervous systems (Bredt *et al.*, 1990). The biological activity of EDRF has been attributed mainly to NO (Moncada *et al.*, 1988), which therefore has been considered the most likely candidate for the NANC transmitter. However, some differences between the vascular and NANC endogenous nitrate systems have been reported (Gillespie & Sheng, 1989; Gibson *et al.*, 1990; Toda & Okamura, 1990). If the two systems are the same, then drugs known to influence EDRF/NO function should show parallel effects on the endogenous nitrate NANC system; indeed, such evidence would be necessary to establish NO as a neurotransmitter. Hydroquinone has been used frequently as an inhibitor of EDRF/NO relaxations of vascular smooth muscle (Furchgott, 1984) where it might act either as a free radical scavenger (Griffith *et al.*, 1984) or as a generator of superoxide anions (Moncada *et al.*, 1986). However, its effects on NANC responses have been less widely studied and results have been varied; Gillespie & Sheng (1990) found that hydroquinone produced a small reduction of NANC relaxations of the rat anococcygeus but had no effect on the bovine retractor penis. The object of the present study was to investigate in more detail the interaction between hydroquinone and NANC nerves by determining its effects on three other tissues possessing an endogenous nitrate NANC system: the mouse anococcygeus (Gibson *et al.*, 1990), guinea-pig trachea (Tucker *et al.*, 1990; Li & Rand, 1991), and the rat gastric fundus (Li & Rand, 1990). In addition, an attempt was made to determine whether the effect of hydroquinone resulted from production of superoxide anions. Some of the work has been presented to the British Pharmacological Society (Gibson & Mirzazadeh, 1989a; Hobbs *et al.*, 1991).

Methods

Mouse anococcygeus

Male mice (LACA; 25–35 g) were killed by stunning and exsanguination. The paired anococcygeus muscles were dissected, joined by the ventral bar, and set up in series in 2 ml

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glass organ baths containing Krebs bicarbonate buffer (mM: NaCl 118.1, KCl 4.7, $MgSO_4$ 1.0, KH_2PO_4 1.0, $CaCl_2$ 2.5, $NaHCO_3$ 25.0 and glucose 11.1) which was maintained at 37°C and gassed continously with 95% O_2:5% CO_2. A resting tension of 200–400 mg was placed on the tissues and changes in tension recorded with a Grass FTO3 force-displacement transducer attached to a Graphtec pen-recorder (WR 3101). Muscles were allowed to equilibrate for 45 min before beginning the experiment. Field stimulation was applied via two parallel platinum electrodes running down either side of the tissue; these were attached to a Grass S48 stimulator (0.5 ms pulse width; 70 V). To observe relaxations to field stimulation in the anococcygeus it is necessary to raise muscle tone and negate contractions due to release of noradrenaline from sympathetic nerves. Tone was raised with 50 μM carbachol in all cases; sympathetic responses were prevented by including 1 μM phentolamine in the Krebs solution and by pre-incubating each muscle with 30 μM guanethidine for 10 min during the 45 min equilibration period. Field stimulation (10 Hz; 10 s trains every 100 s), under these conditions, produced NANC relaxations which were reduced by 95% in the presence of 50 μM L-NOARG (Gibson *et al.*, 1990).

To record relaxations to drugs, tone was first raised with 50 μM carbachol. When carbachol-induced tone had reached a plateau (usually within 3 min), the relaxant drug was added to the organ bath. The response was calculated as the peak % reduction of carbachol-induced tone occurring within 4 min of addition of the relaxant drug; if no peak occurred within 4 min, the response at that time was used. Both carbachol and the relaxant were then washed out of the organ bath and the muscle allowed to rest for 20 min before tone was raised again.

Guinea-pig trachea

Male guinea-pigs (Dunkin-Hartley; 600–900 g) were stunned, exsanguinated and the trachea excised. Surrounding connective tissue was removed and tranverse strips consisting of two adjacent cartilage rings were set up for the recording of isometric tension responses and application of field stimulation as described above (resting tension 0.5 g). All experiments were conducted in the presence of atropine (1 μM), propranolol (1 μM), indomethacin (2 μM) and α-chymotrypsin (2 u ml^{-1}). Muscle tone was raised with the thromboxane-mimetic U46619 (50 nM). Again, relaxations were calculated as the % reduction of tone occurring within 4 min of application of relaxant drug or field stimulation.

Rat gastric fundus

Male rats (Wistar; 250–350 g) were stunned, exsanguinated and their stomachs removed. Longitudinal muscle strips approximately 10 mm long by 2 mm wide were prepared from the fundus by cutting parallel to the greater curvature and set up for the measurement of isometric tension responses and application of field stimulation as described above (resting tension 0.5 g). All experiments were conducted in the presence of atropine (2 μM) and guanethidine (5 μM). Muscle tone was raised with U46619 (100 nM). Relaxations were calculated as % reduction of tone, occurring within 4 min of application of relaxant drug or field stimulation.

In all three of the above preparations, hydroquinone (or SOD) was added to the bath 10 min before the relaxing stimulus. Only one concentration of relaxant, or one train of field stimulation, was applied on each occasion that tone was raised with carbachol or U46619.

Rabbit aorta

Male rabbits (New Zealand White; 2.5–3.5 kg) were killed by overdose of pentobarbitone (60 mg kg^{-1}, i.v.). The thoracic aorta was dissected out and cleared of connective tissue. Intact rings, 3–4 mm in diameter, were set up for the recording of isometric tension responses as described above (resting tension 2 g). Rings were pre-contracted with phenylephrine (500 nM). Methacholine (0.1–10 μM) was administered cumulatively to elicit concentration-dependent relaxations.

Chemiluminescent studies

Superoxide anions were detected by chemiluminescence (Cherry *et al.*, 1990). Two ml Krebs solution containing 250 μM lucigenin was added to clear polystyrene cuvettes. These were positioned in an LKB-Wallac 1250 luminometer in which they were maintained at 37°C and gassed continuously with 95% O_2:5% CO_2. The chemiluminescent signal (measured in mV) was monitored continuously by digital read-out, and in analogue form, by an attached pen-recorder. After a 15 min equilibration period, increasing concentrations of hydroquinone were added to the cuvette via an auto-injector (LKB 1250-104). In some experiments, a pair of anococcygeus muscles from a single mouse were minced and added to the cuvette prior to the hydroquinone. A new cuvette, and tissue where appropriate, was used for each new concentration of hydroquinone. The ability of the system to detect superoxide anions was determined by use of xanthine:xanthine oxidase. Here 0.07 u ml^{-1} xanthine oxidase was included with 250 μM lucigenin in 2 ml Krebs solution in the cuvette; increasing concentrations of xanthine were then added via the auto-injector. In certain experiments superoxide dismutase (SOD; 50 u ml^{-1}) or hydroquinone (100 μM) was added to the cuvette 10 min before the xanthine.

Statistics

Results are expressed as mean ± s.e.mean. Statistical analysis was by Student's *t* test (unpaired); a probability (*P*) value of <0.05 was taken to indicate statistical significance.

Drugs

All drugs used in this study were dissolved in distilled water, except xanthine oxidase, which was in 2.3 M $(NH_4)_2SO_4$ buffer, and indomethacin which was in 0.5% Na_2CO_3. NO solutions and concentrations were prepared and calculated as described previously (Gibson & Mirzazadeh, 1989b).

Drugs used were: atropine sulphate (Sigma); α-chymotrypsin (Type I-S; Sigma); carbachol (BDH); guanethidine sulphate (Ciba); hydroquinone (Sigma); hydroxylamine hydrochloride (Sigma); indomethacin (Sigma); lucigenin (*bis*-methylacridinium nitrate; Sigma); methacholine chloride (Sigma); nitric oxide (99%; BDH); pentobarbitone sodium (Sigma); phentolamine mesylate (Ciba); phenylephrine hydrochloride (Sigma); propranolol hydrochloride (ICI); sodium azide (Sigma); sodium nitroprusside (Sigma); U46619 (9,11-dideoxy methanolepoxy-9α, 11α-prostaglandin $F_{2\alpha}$) (Sigma); xanthine (Sigma); xanthine oxidase (Sigma).

Results

The effect of hydroquinone on nitrovasodilator- and NANC-induced relaxations

Mouse anococcygeus As described previously (Gibson & Mirzazadeh, 1989b; Gibson *et al.*, 1990), carbachol-induced tone of the mouse anococcygeus showed graded relaxations in the presence of NO (3–30 μM), hydroxylamine (5–200 μM), sodium nitroprusside (SNP; 0.01–10 μM) and sodium azide (1–100 μM). Concentrations of each drug which reduced tone by between 30–80% were chosen to study the effect of hydroquinone (see control columns in Figure 2). To establish an effective concentration of hydroquinone it was first tested against NO. Relaxations induced by 15 μM NO were reduced, in a concentration-dependent manner, by hydroquinone (10–100 μM; Figure 1); the inhibitory effect of each concentration of hydroquinone was easily reversed by washout. Hydro-

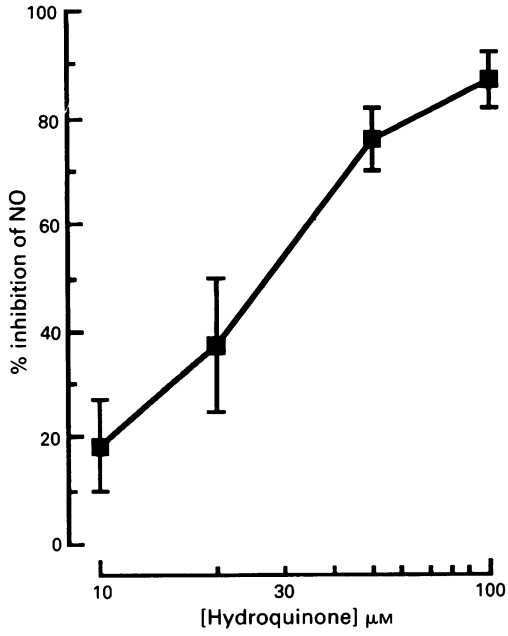

Figure 1 Concentration-response curve for hydroquinone causing inhibition of nitric oxide (NO; 15 μM)-induced relaxations of the mouse anococcygeus muscle. Tone was raised with 50 μM carbachol; hydroquinone was added 10 min prior to the NO. Each point represents the mean from at least 6 individual muscle preparations; s.e.mean shown by vertical bars.

quinone, 100 μM, which reduced responses to NO by about 85%, had no effect on relaxations induced by hydroxylamine (10 μM), SNP (1 μM) or sodium azide (20 μM; Figure 2). Field stimulation (10 Hz; 20 s trains) caused reproducible NANC relaxations which have previously been shown to be blocked by L-NOARG (Gibson *et al.*, 1990); these relaxations were, however, unchanged in the presence of 100 μM hydroquinone (Figure 2). Although not shown, we also studied the effect of hydroquinone with 20 s trains at 1, 2 and 5 Hz ($n = 2$ in each case) but, again, no change in response was observed.

Guinea-pig trachea In these experiments, in addition to propranolol (1 μM), indomethacin (2 μM) and α-chymotrypsin

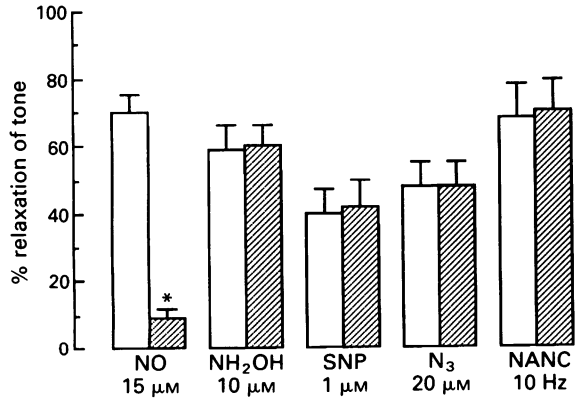

Figure 2 Histogram showing the effect of hydroquinone (100 μM) on relaxations of the mouse anococcygeus in response to nitric oxide (NO), hydroxylamine (NH_2OH), sodium nitroprusside (SNP), sodium azide (N_3) and non-adrenergic, non-cholinergic field stimulation (NANC). Muscle tone was raised with 50 μM carbachol; hydroquinone was in contact with the tissue for 10 min before application of the relaxing stimulus. Open columns, control; hatched columns, in the presence of 100 μM hydroquinone. Each column represents the mean from at least 6 individual muscle preparations; s.e.mean shown by vertical bars. * $P < 0.05$, significant reduction of response. Hydroquinone reduced responses to NO but not to NANC stimulation or other nitrovasodilators.

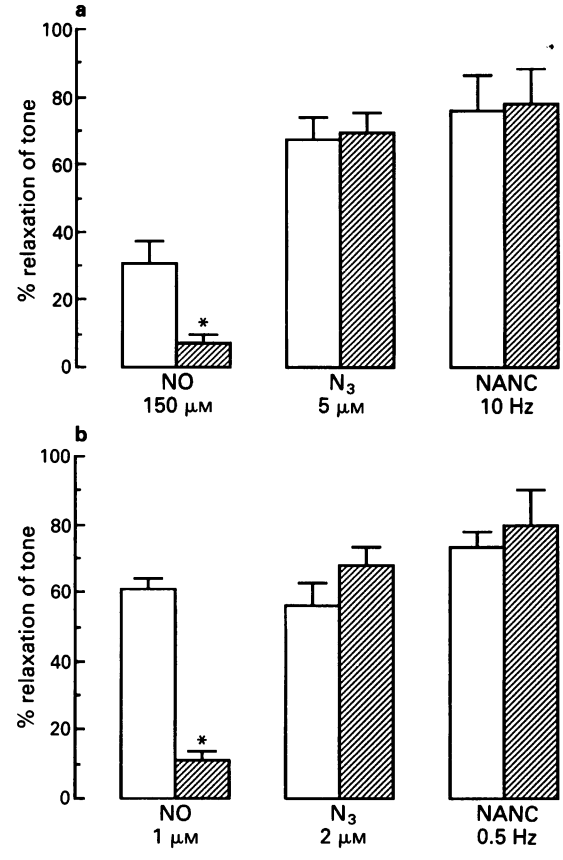

Figure 3 Histograms showing the effect of hydroquinone (100 μM) on relaxations of the guinea-pig trachea (a) and rat gastric fundus (b) in response to nitric oxide (NO), sodium azide (N_3) and non-adrenergic, non-cholinergic field stimulation (NANC). Muscle tone was raised with U46619 (50 nM in trachea; 100 nM in fundus); hydroquinone was in contact with tissue for 10 min before application of the relaxing stimulus. Open columns, control; hatched columns, in the presence of 100 μM hydroquinone. Each column represents the mean of at least 6 individual muscle preparations; s.e.mean shown by vertical bars. * $P < 0.05$, significant reduction of response. Hydroquinone reduced responses to NO in both tissues but not those to NANC stimulation or sodium azide.

(2 u ml^{-1}), atropine (1 μM) was included in the Krebs solution to prevent any contractile effects due to released acetylcholine. For this reason, we could not use carbachol to raise tone as in our original experiments (Tucker *et al.*, 1990). However, field stimulation (10 Hz; 30 s trains) produced clear NANC relaxations of U46619-induced tone, which were inhibited by 90% in the presence of 100 μM L-NOARG (Hobbs *et al.*, 1991). U46619-induced tone was also reduced by NO (3–300 μM) and sodium azide (0.5–100 μM). Hydroquinone (100 μM) reduced responses to NO (150 μM) by 75% (Figure 3a), but had no effect on those to sodium azide (5 μM) or NANC field stimulation.

Rat gastric fundus U46619 (100 nM)-induced tone in rat gastric fundus was reduced by NANC field stimulation (0.5 Hz; 15 s trains); these relaxations were reduced by 50% in the presence of 50 μM L-NOARG, confirming the involvement of an endogenous nitrate (Li & Rand, 1990). NO (1–20 μM) and sodium azide (0.5–10 μM) also reduced U46619-induced tone. Hydroquinone (100 μM) reduced relaxations to 1 μM NO by 85% (Figure 3b), but had no effect on those to sodium azide (2 μM) or NANC field stimulation.

Investigation of the mechanism of action of hydroquinone

Hydroquinone on methacholine-induced relaxations of rabbit aorta To ensure that hydroquinone was capable of inhibiting

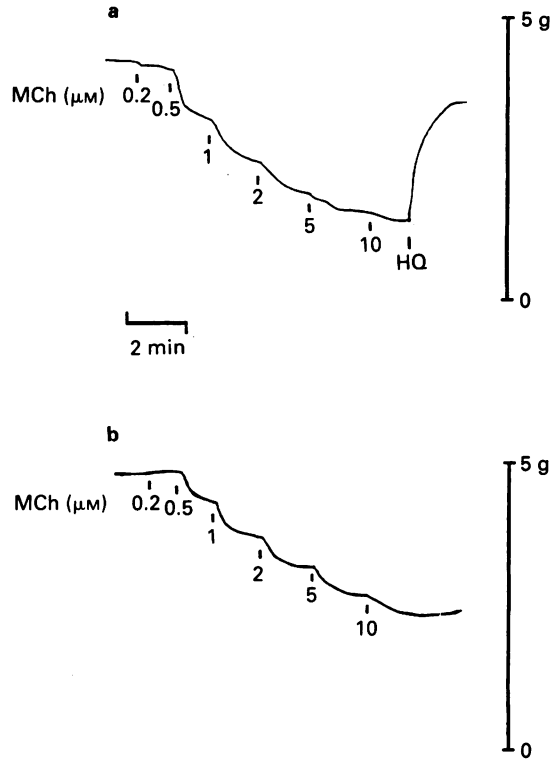

Figure 4 Relaxation of a rabbit aortic ring in response to cumulative additions of methacholine (MCh, a and b) and its reversal by hydroquinone (HQ; 100 μM; a). Muscle tone was raised with 500 nM phenylephrine. The trace is representative of similar results obtained in 10 out of 10 tissues.

endogenously-released nitrate in our system, we investigated its effect on rabbit aortic rings. In rings pre-contracted with phenylephrine (500 nM), methacholine (0.1–10 μM) caused concentration-related relaxations; these were abolished by gently rubbing the luminal surface with cotton wool and were therefore deemed to be due to the release of EDRF. Methacholine (10 μM) reduced tone by approximately 60%; hydroquinone (100 μM) produced a rapid reversal of this relaxation (Figure 4).

The effect of prior field stimulation on the inhibitory effect of hydroquinone It was possible that the passage of current through the Krebs solution might lead to breakdown of hydroquinone. However, the inhibitory effect of 100 μM hydroquinone on NO (15 μM)-induced relaxations of the mouse anococcygeus was still observed after prior exposure to a 10 s train of field stimulation (10 Hz; Figure 5).

The role of superoxide anions The involvement of superoxide anions in the inhibitory effects of hydroquinone was investigated in two series of experiments. In the first, relaxations were obtained in the presence and absence of SOD (50 u ml^{-1}). By itself, SOD had no effect on relaxations induced by 15 μM NO (control relaxation = 54 ± 5%; with SOD = 54 ± 9%; $n = 40$; $P > 0.05$) or 10 Hz NANC stimulation (control relaxation = 55 ± 9%; with SOD = 54 ± 9%; $n = 6$; $P > 0.05$). In addition, the inhibitory effect of 100 μM hydroquinone on relaxations induced by NO was unchanged in the presence of SOD (control inhibition = 91 ± 6%; with SOD = 96 ± 4%; $n = 6$; $P > 0.05$).

In the second series of experiments, superoxide anion generation was assessed by chemiluminescence. In the presence of xanthine oxidase (0.07 u ml^{-1}) and lucigenin (250 μM), the addition of xanthine (10–500 μM) caused a concentration-related generation of superoxide anions, detected by an increase in the chemiluminescent signal; the time course of the effect of 100 μM xanthine and the peak increase produced by

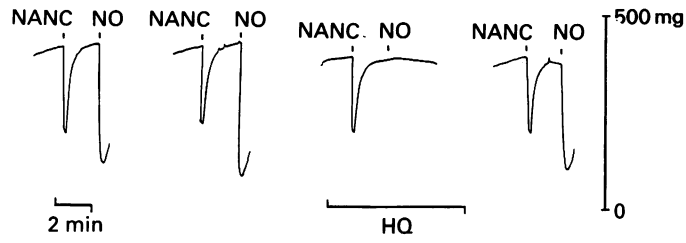

Figure 5 The effect of hydroquinone (HQ; 100 μM) on relaxations of a mouse anococcygeus muscle induced by non-adrenergic, non-cholinergic field stimulation (NANC; 10 Hz; 10 s train) and nitric oxide (NO; 15 μM). Muscle tone was raised by carbachol (50 μM) which was washed out of the bath after each dose of NO and the muscle allowed to rest for 20 min before tone was raised again. Hydroquinone was added 10 min before NANC stimulation. Hydroquinone reduced responses to NO, but not NANC stimulation, even after prior exposure to field stimulation. The trace is representative of similar results obtained in 5 out of 5 tissues.

each concentration of xanthine are shown in Figure 6. SOD (50 u ml^{-1}) produced almost complete inhibition of superoxide anion chemiluminescence (Figure 7). Hydroquinone (10–100 μM) produced no generation of superoxide anions; however, 100 μM hydroquinone greatly reduced the chemiluminescence generated by 500 μM xanthine (Figure 7). Essentially, similar results were obtained when the above experiments were repeated with the inclusion of minced anococcygeus tissue in the cuvette, in which xanthine (500 μM):xanthine oxidase chemiluminescence was reduced by

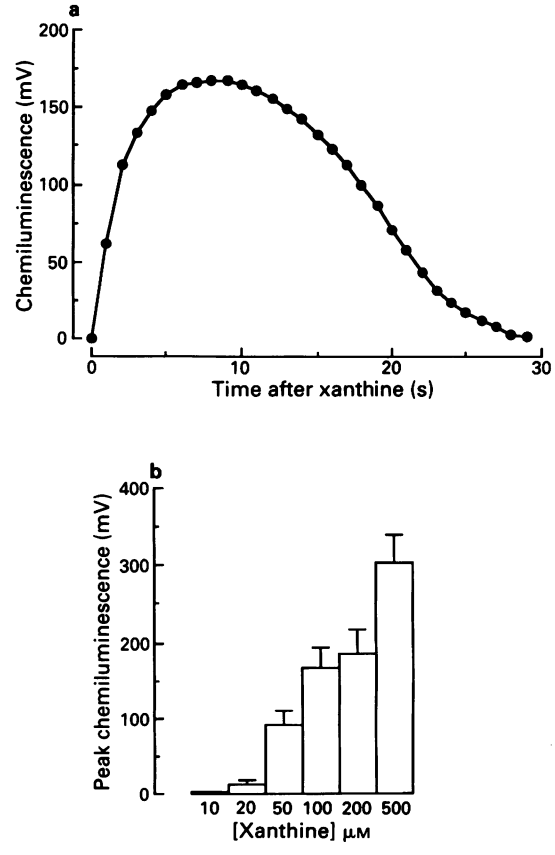

Figure 6 Time-course (a) and peak increase (b) of the chemiluminescent signal generated by xanthine:xanthine oxidase. A constant concentration of xanthine oxidase (0.07 u ml^{-1}) was used; in (a) 100 μM xanthine and in (b) increasing concentrations of xanthine were added to induce the production of superoxide anions. Each point represents the mean of at least 6 experiments; s.e.mean shown by vertical bars in (b) and omitted in (a) for clarity.

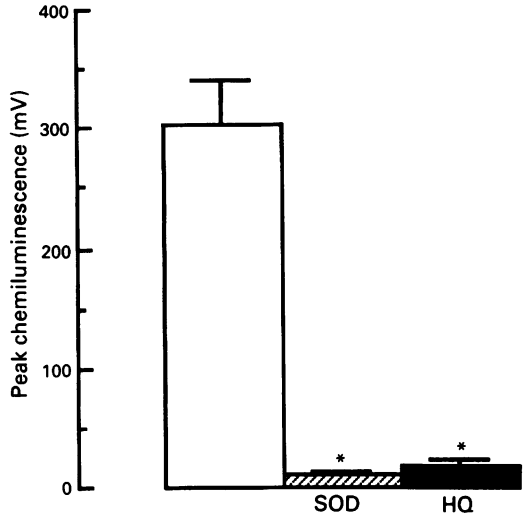

Figure 7 Histogram showing the effect of superoxide dismutase (SOD; 50 u ml^{-1}) and hydroquinone (HQ; 100 μM) on the peak chemiluminescent signal generated by xanthine (500 μM):xanthine oxidase (0.07 u ml^{-1}). SOD and HQ were added 10 min before xanthine. Each column represents the mean of at least 6 individual observations; s.e.mean shown by vertical bars. * $P < 0.05$, significant reduction of chemiluminscent signal.

93 ± 2% ($n = 5$) in the presence of SOD (50 u ml^{-1}) and by 96 ± 1% ($n = 5$) in the presence of hydroquinone (100 μM).

Discussion

NO-synthase inhibitors have been shown to block NANC relaxations in the mouse anococcygeus (Gibson *et al.*, 1990), guinea-pig trachea (Tucker *et al.*, 1990; Hobbs *et al.*, 1991; Li & Rand, 1991) and rat gastric fundus (Li & Rand, 1990; Boeckxstaens *et al.*, 1991); the inhibition is stereoselective and is reversed by L-, but not D-, arginine. Further, in the anococcygeus the relaxations to both NANC stimulation and nitrovasodilators are selectively reduced by the guanylate cyclase inhibitor N-methylhydroxylamine (Gibson & Mirzazadeh, 1989b). Such observations have led to the proposal that, in each tissue, endogenous nitrates contribute to the NANC response. Since the system is similar to that which generates EDRF/NO (Moncada *et al.*, 1988), and since NO-synthase has been detected in nerves (Bredt *et al.*, 1990), NO has been considered as the putative NANC transmitter. If NO is the actual transmitter substance released by the NANC nerves it would be expected that drugs which influence the actions of NO would exert a similar effect on the NANC response. However, in the present study, hydroquinone produced marked inhibition of relaxations to NO in all three tissues but had no effect on those to NANC stimulation. Hydroquinone also failed to influence NANC relaxations in the bovine retractor penis (Gillespie & Sheng, 1990), but produced a small inhibitory effect in the rat anococcygeus; why the rat anococcygeus should differ from the other tissues in its response to hydroquinone is not known. It is unlikely that lack of penetration of hydroquinone into the tissue can account for its differentiation between exogenous and endogenous nitrate in the present study because it produced the expected reversal of EDRF/NO-induced relaxations of rabbit aorta (Furchgott, 1984). Similarly, breakdown of hydroquinone by the current passing through the Krebs solution during field stimulation cannot be an explanation, since the activity of hydroquinone persisted after prior exposure to field stimulation. Consequently, the observed lack of parallelism may suggest that free NO is not liberated by the NANC nerves. While there is now strong evidence that an endogenous nitrate system contributes to NANC relaxations in several tissues (see Introduction), the precise nature of the substance released by the nerves remains to be established. Gillespie & Sheng (1989) found that NO and inhibitory factor from bovine retractor penis (the putative NANC transmitter) differed in their ability to penetrate red blood cells and suggested that the NANC nerves might release a NO-containing, or NO-generating, molecule rather than NO itself. Our findings would support this proposal since hydroquinone not only differentiated between NO and NANC stimulation, but also between NO and other nitrovasodilators. In this respect, hydroquinone appears to be more useful than haemoglobin as a probe to investigate possible roles for NO, since haemoglobin is known to bind and inactivate not just NO but also a wide range of nitrovasodilators (Colter & Quastel, 1950; Mittal *et al.*, 1978; Martin *et al.*, 1985; Laurence & Bennett, 1987). Bult *et al.* (1990) reported the release of NO from dog ileocolonic junction following NANC nerve stimulation and used this to support the view that NO is the NANC transmitter. However, as pointed out by Ignarro *et al.* (1990b), the detected NO could equally be coming from other sources having been generated or liberated by the actual transmitter. Even in the case of EDRF there have been recent reports that some nitroso compounds more closely mimic the properties of EDRF than does NO (Graser *et al.*, 1990; Myers *et al.*, 1990; Rubanyi *et al.*, 1990), and it may be that a similar substance is liberated from the NANC nerves. Several physiologically-relevant S-nitrosothiols have recently been shown to relax smooth muscle (Kowaluk & Fung, 1990), although interpretation of results is complicated by their differing abilities to liberate free NO in solution. Nevertheless, these and other S-nitrosothiols are clearly of interest in terms of the NANC transmitter and require closer investigation; so too does hydroxylamine, which has been proposed as a possible intermediate in the generation of NO from L-arginine (DeMaster *et al.*, 1989) and which, like NANC stimulation, was resistant to hydroquinone. Of course, the possibility that free NO is the transmitter, and that there is some other reason for the differential effect of hydroquinone, cannot be dismissed; until such a reason is found the precise nature of the NANC transmitter remains in doubt.

It has been suggested that hydroquinone inhibits EDRF/NO relaxations of vascular smooth muscle by acting either as a free radical scavenger (Griffith *et al.*, 1984) or as a generator of superoxide anions (Moncada *et al.*, 1986). Our observations with SOD and with the chemiluminescent assay suggest that superoxide anion generation cannot explain the inhibitory effect of hydroquinone on NO in the present study. If hydroquinone was acting as a superoxide anion generator its inhibitory effects on NO should have been blunted by SOD. However, the inhibitory potency of hydroquinone was unchanged in the presence of SOD; indeed, superoxide anions do not seem to play an important role in our system since SOD had no effect on relaxations induced by NO or NANC stimulation. This was confirmed by the chemiluminescent studies in which no superoxide anion chemiluminescence was present under control conditions and, further, no superoxide anion generation was induced by hydroquinone. The assay was capable of detecting superoxide anions since concentration-related increases in chemiluminescence were obtained with xanthine:xanthine oxidase, an effect prevented by SOD. However, while hydroquinone did not generate superoxide anions it acted like SOD, and reduced the chemiluminescent response to xanthine:xanthine oxidase, which would be consistent with it acting as a free-radical scavenger. The lack of effect of SOD on the NANC relaxation is similar to the observations of Toda & Okamura (1990) on cerebral arteries; again, these authors suggested the substance released by the NANC nerves may be an NO-related compound that is resistant to superoxide anions, rather than NO itself.

In conclusion, hydroquinone blocks relaxations induced by NO, but not NANC nerve stimulation or other nitrovasodilator drugs, in three tissues in which NANC relaxations involve an endogenous nitrate. Free radical scavenging, rather than superoxide anion generation, appears to be the mecha-

nism of action of hydroquinone. In the absence of other satisfactory explanations, the differential effect of hydroquinone on NO- and NANC-induced relaxations may suggest that the transmitter released by the NANC nerves is not free NO, but may be an NO-containing, or NO-generating, substance (Gillespie & Sheng, 1989).

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