British Journal of Pharmacology (1997) 121, 813–819

Hydrogen peroxide-induced impairment of reactivity in rat isolated aorta: potentiation by 3-amino-1,2,4-triazole

Kousar B. Mian & [1]William Martin

Clinical Research Initiative, West Medical Building, University of Glasgow, Glasgow, G12 8QQ

1 In this study the impairment induced by hydrogen peroxide of vascular reactivity and the role of endogenous catalase in protection against this impairment was assessed in isolated rings of rat aorta.

2 Incubation with hydrogen peroxide at 1 mM, but not at 0.1 mM, for 15, 30 or 60 min followed by washout depressed, in a time-dependent manner, the subsequent ability of endothelium-containing and endothelium-denuded rings to contract to phenylephrine.

3 Incubation with 3-amino-1,2,4-triazole (50 mM, 90 min, followed by washout) to inhibit endogenous catalase had no effect by itself on subsequent phenylephrine-induced contraction. However, pretreatment with 3-amino-1,2,4-triazole did lead to a profound enhancement of the ability of hydrogen peroxide (1 mM, present for the final 30 min of the 90 min incubation, followed by washout) to depress phenylephrine-induced contraction in both endothelium-containing and endothelium-denuded rings.

4 Incubation with hydrogen peroxide at 1 mM, but not at 0.1 mM, for 15, 30 or 60 min followed by washout inhibited, in a time-dependent manner, the subsequent ability of acetylcholine (10 nM – 3 μM) to induce endothelium-dependent relaxation. Furthermore, incubation with hydrogen peroxide 1 mM (30 min, followed by washout) also inhibited relaxation induced by glyceryl trinitrate (1 – 100 nM) or isoprenaline (10 nM – 3 μM) in endothelium-denuded rings.

5 Incubation with 3-amino-1,2,4-triazole (50 mM, 90 min, followed by washout) had no effect by itself on relaxation induced by acetylcholine, glyceryl trinitrate or isoprenaline. In contrast, pretreatment with 3-amino-1,2,4-triazole led to profound enhancement of the ability of hydrogen peroxide (1 mM, present for final 30 min of the 90 min incubation) to block relaxation to acetylcholine, glyceryl trinitrate or isoprenaline.

6 On the basis of the actions of 3-amino-1,2,4-triazole, it is likely that endogenous catalase plays an important role in the protection of vascular reactivity of rat aorta against oxidant damage by high (1 mM) but not lower (0.1 mM) concentrations of hydrogen peroxide. The data are consistent with the promotion of non-selective damage to the vascular smooth muscle cells by hydrogen peroxide, but endothelial damage may also be sustained.

Keywords: Hydrogen peroxide; catalase; 3-amino-1,2,4-triazole; rat aorta; nitric oxide; acetylcholine; glyceryl trinitrate; isoprenaline

Introduction

The powerful oxidant, hydrogen peroxide, is produced as a by-product of many cellular reactions. It is generated, for example, during mitochondrial respiration (Dionisi *et al*., 1975), during the oxygen burst by neutrophils (Setty *et al*., 1984), from the activity of a number of enzymes including glucose oxidase, xanthine oxidase and cytochrome P_{450} (Servanian & Hochstein, 1985) and from the autoxidation of glutathione (Misra, 1974), ascorbate (Chance, 1950), catecholamines and flavins (Freeman & Crapo, 1982). The effects of hydrogen peroxide on the vasculature are complex, with low concentrations (1 – 100 μM) stimulating release of prostacyclin (Harlan & Callahan, 1984) and nitric oxide (Furchgott, 1991; Mian & Martin, 1995) from endothelial cells and higher concentrations (> 100 μM) leading to endothelial damage, resulting in morphological alterations (Hinshaw *et al*., 1989), neutrophil adhesion (Lewis *et al*., 1988) and vascular leakage (Berman & Martin, 1993). Low concentrations of hydrogen peroxide also produce vasodilatation by direct stimulation of smooth muscle guanylate cyclase (Burke & Wolin, 1987; Mian & Martin, 1995) but higher concentrations impair contractility by oxidative damage (Iesaki *et al*., 1994).

The enzyme catalase is present in many cell types and acts as a high capacity system for the removal of hydrogen peroxide (Halliwell, 1982; Cheeseman & Slater, 1993). This enzyme is also known to play an important role in regulating the vasodilator actions of certain agents. Specifically, we recently found (Mian & Martin, 1995) that 3-amino-1,2,4-triazole, which inhibits catalase (Heim *et al*., 1956; Margoliash & Novogrodsky, 1958), selectively blocks vasodilatation induced by sodium azide and hydroxylamine in rat aorta, consistent with the proposition that these nitrovasodilators are converted to nitric oxide by this enzyme (Katsuki *et al*., 1977; Waldman & Murad, 1987). The object of the present study was to make use of 3-amino-1,2,4-triazole to determine if endogenous catalase protects vascular function in rat aorta from the damaging actions of hydrogen peroxide. A preliminary account of these findings has already been published (Mian & Martin, 1996).

Methods

Preparations of aortic rings

Female Wistar rats of approximately 200 – 250 g were killed by stunning and exsanguination. The thoracic aorta was removed and cut into 2.5 mm wide transverse rings with a razor blade slicing device. In some experiments, the endothelium was removed by locating the aortic ring between two stainless steel hooks, placing a 2 g weight on the bottom hook and gently rubbing the intimal surface with a moist match stick for 10 – 20 s. Endothelial denudation was deemed successful if no relaxation took place in response to acetylcholine (1 μM).

[1] Author for correspondence.

Tension recording

The aortic rings were mounted under 1 g resting tension on stainless steel hooks in 20 ml organ baths maintained at 37°C containing Krebs solution (mM): NaCl 118, KCl 4.8, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 24, and glucose 11, and gassed with 95% O_2 and 5% CO_2. Tension was recorded isometrically by means of Grass FT03C transducers and responses were displayed on a Grass polygraph model 7. Tissues were allowed to equilibrate for 90 min before experiments were carried out, during which time the resting tension was re-adjusted to 1 g, as required.

Experimental protocols

Following induction of submaximal phenylephrine-induced tone in endothelium-containing (phenylephrine 10 nM) and endothelium-denuded (phenylephrine 3 nM) rings of rat aorta, the effects of incubation with hydrogen peroxide (0.1 or 1 mM) for 15, 30 or 60 min, followed by washout, were examined on the subsequent ability of phenylephrine to induce contraction.

The effects of incubation with hydrogen peroxide were also examined on acetylcholine-induced relaxation. In these experiments endothelium-containing rings precontracted with phenylephrine (10 nM) were incubated with hydrogen peroxide (0.1 or 1 mM) for 15, 30 or 60 min. The tissues were then washed out and cumulative concentration-response curves to acetylcholine (10 nM – 3 μM) were constructed to determine if any impairment of endothelium-dependent relaxation had taken place.

The effects of incubation with hydrogen peroxide were also examined on the endothelium-independent relaxations induced by glyceryl trinitrate and isoprenaline. In these experiments endothelium-denuded rings precontracted with phenylephrine (3 – 10 nM) were incubated with hydrogen peroxide (0.1 or 1 mM) for 30 min. The tissues were then washed out and cumulative concentration-response curves to glyceryl trinitrate (1 – 100 nM) and isoprenaline (10 nM – 3 μM) constructed to determine if any impairment of relaxation had taken place.

Use of 3-amino-1,2,4-triazole

In certain experiments, we wished to use 3-amino-1,2,4-triazole, a selective inhibitor of catalase (Heim *et al*., 1956; Margoliash & Novogrodsky, 1958), to inhibit the activity of endogenous catalase. This was done to determine if endogenous catalase exerted a protective action against exogenous hydrogen peroxide.

The effects of incubation with 3-amino-1,2,4-triazole (50 mM, 90 min, followed by washout) either alone or together with hydrogen peroxide (0.1 or 1 mM, present during the final 30 min of 90 min incubation with 3-amino-1,2,4-triazole) were examined on the subsequent ability of: (i) phenylephrine to contract endothelium-containing (phenylephrine 10 nM) and endothelium-denuded (phenylephrine 3 nM) rings; (ii) acetylcholine (10 nM – 3 μM) to relax endothelium-containing rings; and (iii) glyceryl trinitrate (1 – 100 nM) or isoprenaline (10 nM – 3 μM) to relax endothelium-denuded rings.

Drugs

Acetylcholine chloride, 3-amino-1,2,4-triazole, catalase (bovine liver), hydrogen peroxide (30%), phenylephrine hydrochloride and isoprenaline hydrochloride were obtained from Sigma (Poole, Dorset), whilst glyceryl trinitrate was obtained from NAPP Laboratories (Cambridge). All drugs were dissolved and dilutions made in saline (0.9%).

Statistical analysis

Results are expressed as the mean ± s.e.mean for n separate experiments and comparisons were made by one-way analysis of variance followed by Fisher's test. A probability of 0.05 or less was considered significant.

Results

Effects of hydrogen peroxide (0.1 or 1 mM) on phenylephrine-induced contraction

Following induction of phenylephrine (10 nM)-induced contraction (1.3 ± 0.1 g, $n = 12$) in endothelium-containing rings, addition of hydrogen peroxide (0.1 mM) produced powerful long-lasting relaxation (79.5 ± 1.5%, $n = 12$). When the incubation was continued for 15, 30 or 60 min and the tissues washed, the subsequent phenylephrine (10 nM)-induced contraction was unaffected (Figure 1a). Hydrogen peroxide (0.1 mM) also relaxed phenylephrine (3 nM)-contracted (1.2 ± 0.1 g, $n = 10$) endothelium-denuded rings (relaxation 75.1 ± 1.0%, $n = 10$). As with endothelium-containing rings, incubation with hydrogen peroxide (0.1 mM) for 15 to 60 min had no effect on the subsequent phenylephrine-induced contraction (Table 1).

Increasing the concentration of hydrogen peroxide to 1 mM also produced powerful relaxation (96.2 ± 3.5%, $n = 12$) of endothelium-containing rings, but when the incubation was continued for 15, 30 or 60 min followed by washout, the subsequent phenylephrine (10 nM)-induced contraction was inhibited in a time-dependent manner (Figure 1b). Catalase

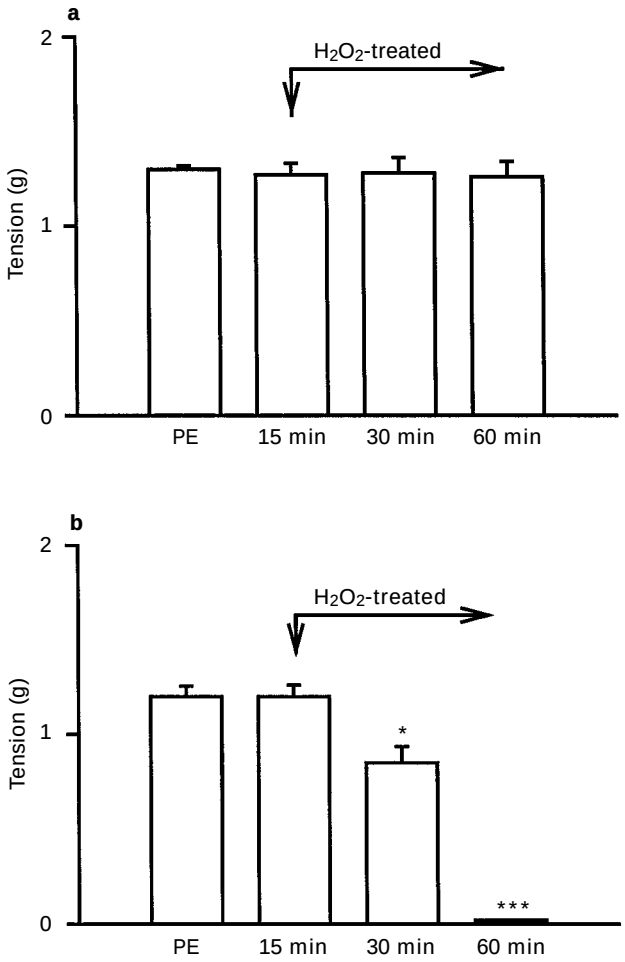

Figure 1 (a) Incubation of endothelium-containing rings of rat aorta with hydrogen peroxide (H_2O_2, 0.1 mM) for 15, 30 or 60 min followed by washout had no effect on subsequent phenylephrine (PE, 10 nM)-induced contraction. (b) Incubation of endothelium-containing rings with H_2O_2 (1 mM) for 15 min followed by washout had no effect on subsequent phenylephrine (10 nM)-induced contraction, but extending the incubation period to 30 or 60 min followed by washout significantly depressed contraction. Each column is the mean ± s.e.mean of 6 – 12 observations. $^*P < 0.05$ and $^{***}P < 0.001$ indicate a significant difference from rings receiving phenylephrine only.

Table 1 Effects of exposure to hydrogen peroxide and 3-amino-1,2,4-triazole (AT) alone and in combination followed by washout on the subsequent ability of endothelium-denuded rings to contract to phenylephrine (3 nM)

Treatment	*Contraction* (g)
Control	1.2±0.1
H_2O_2 0.1 mM (15 min)	1.3±0.1
H_2O_2 0.1 mM (30 min)	1.2±0.2
H_2O_2 0.1 mM (60 min)	1.3±0.1
H_2O_2 1 mM (15 min)	1.1±0.1
H_2O_2 1 mM (30 min)	0.6±0.1*
H_2O_2 1 mM (60 min)	0.1±0.1***
Control	1.3±0.1
AT 50 mM (90 min)	1.2±0.1
H_2O_2 1 mM (30 min)	0.5±0.2*
AT + H_2O_2	0.1±0.1***

Contractions are given in g tension and represent the mean±s.e.mean of 6–10 observations. *$P<0.05$ and ***$P<0.001$ indicate significant differences from contractions on control rings.

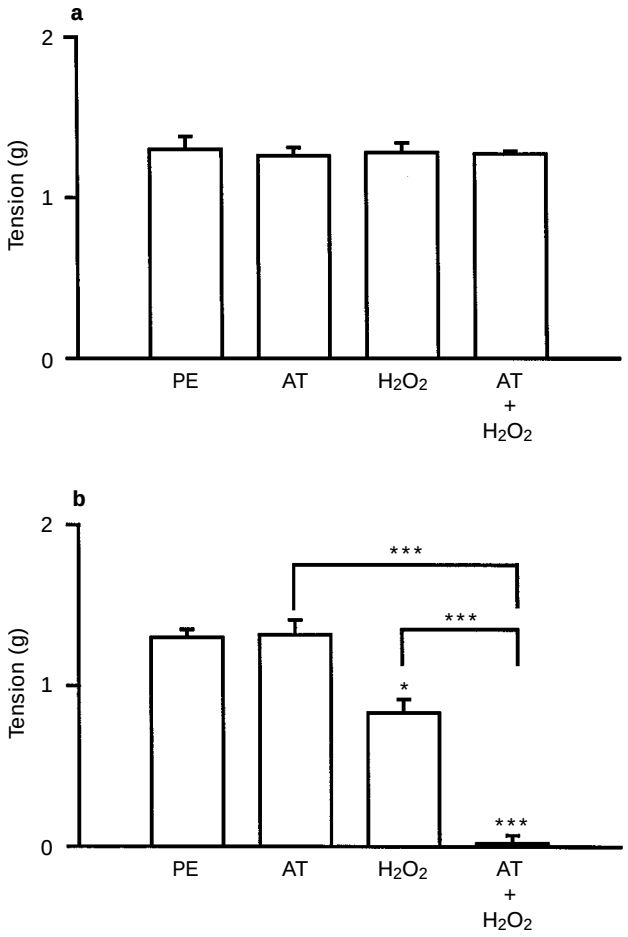

Figure 2 (a) Incubation with the inhibitor of catalase, 3-amino-1,2,4-triazole (AT, 50 mM, 90 min, followed by washout) alone, incubation with hydrogen peroxide (H_2O_2, 0.1 mM, 30 min, followed by washout) alone, or the combined treatment with 3-amino-1,2,4-triazole (50 mM, 90 min) and H_2O_2 (0.1 mM, present during the final 30 min of the 90 min incubation, followed by washout) had no effect on phenylephrine (PE, 10 nM)-induced contraction in endothelium-containing rings of rat aorta. (b) However, incubation with H_2O_2 (1 mM, 30 min, followed by washout) significantly depressed phenylephrine-induced contraction. Furthermore, contraction was depressed further if H_2O_2 (1 mM) was present during the final 30 min of the 90 min incubation with 3-amino-1,2,4-triazole. Each column is the mean±s.e.mean of 6–10 observations. *$P<0.05$ and ***$P<0.001$ indicate a significant difference from rings receiving phenylephrine only or between groups joined by brackets.

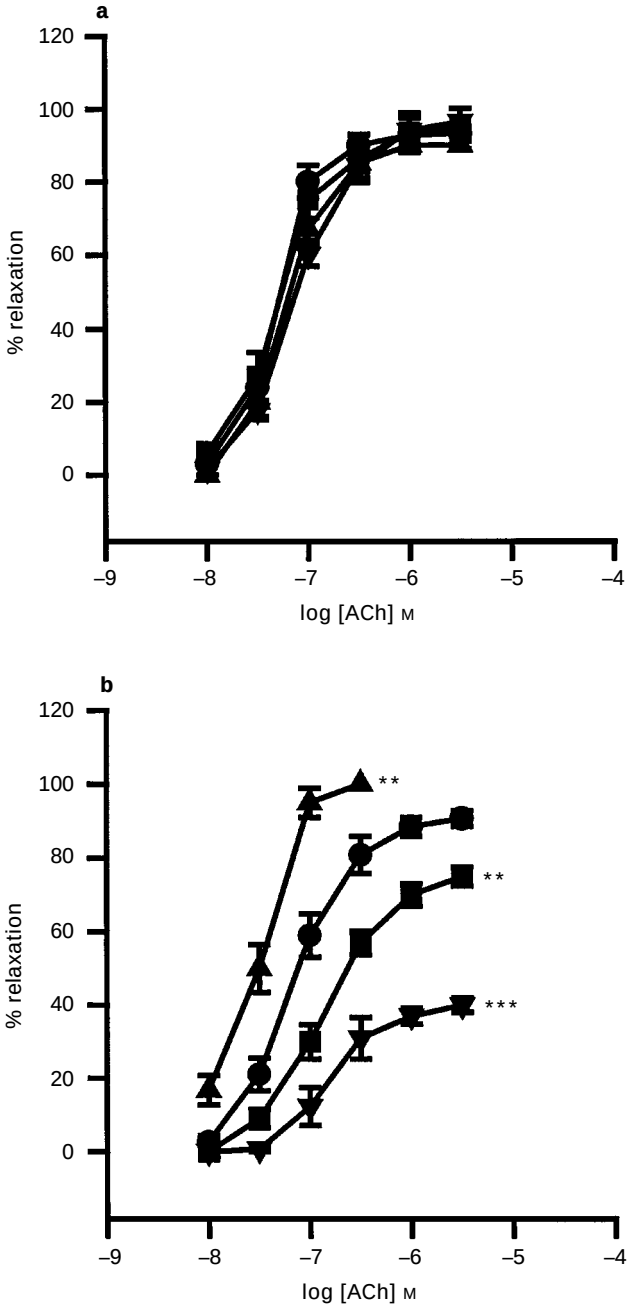

Figure 3 (a) Concentration-response curves showing relaxation to acetylcholine (ACh, ●) on phenylephrine-contracted endothelium-containing rings of rat aorta and on rings following incubation with hydrogen peroxide (H_2O_2, 0.1 mM) for 15 min (▲), 30 min (■) or 60 min (▼) followed by washout. (b) Concentration-response curves showing relaxation to acetylcholine (●) and potentiation of this following incubation with H_2O_2 (1 mM) for 15 min followed by washout (▲). Furthermore, incubation with H_2O_2 (1 mM) for 30 min (■) or 60 min (▼) followed by washout resulted in significant blockade of acetylcholine-induced relaxation. Each point is the mean and vertical lines s.e.mean of 5–12 observations. **$P<0.005$ and ***$P<0.001$ indicate a significant difference from maximal relaxations obtained in untreated rings.

(1000 u ml^{-1}) inhibited the ability of hydrogen peroxide to produce relaxation, as previously found (Mian & Martin, 1995). However, it failed to reverse the depression of phenylephrine-induced contraction seen following treatment with hydrogen peroxide for 60 min (contraction depressed to 0.1±0.1 g, $n=6$). The time-dependent depression by hydrogen peroxide (1 mM) of subsequent contraction to phenylephrine was also seen in endothelium-denuded rings (Table 1).

Effects of 3-amino-1,2,4-triazole on the ability of hydrogen peroxide to impair phenylephrine-induced contraction

Incubation of endothelium-containing rings with 3-amino-1,2,4-triazole (50 mM, 90 min, followed by washout) to inhibit endogenous catalase, had no effect by itself on the subsequent phenylephrine (10 nM)-induced contraction (Figure 2a). Furthermore, including hydrogen peroxide (0.1 mM) for the final 30 min of the 90 min incubation with 3-amino-1,2,4-triazole followed by washout still failed to affect the subsequent phenylephrine-induced contraction. In contrast, including a higher concentration of hydrogen peroxide (1 mM) for the final 30 min of the 90 min incubation with 3-amino-1,2,4-triazole followed by washout led to a greater depression of phenylephrine (10 nM)-induced contraction than obtained with hydrogen peroxide (1 mM) alone (Figure 2b). In endothelium-denuded rings also, 3-amino-1,2,4-triazole potentiated the ability of hydrogen peroxide at 1 mM to depress phenylephrine (3 nM)-induced contraction (Table 1). However, 3-amino-1,2,4-triazole did not influence the inability of hydrogen peroxide (0.1 mM) to affect subsequent phenylephrine-induced contraction on these rings.

Effects of hydrogen peroxide (0.1 or 1 mM) on acetylcholine-induced relaxation

Incubation of endothelium-containing rings with hydrogen peroxide (0.1 mM) for 15, 30 or 60 min followed by washout had no effect on subsequent acetylcholine (10 nM – 3 μM)-induced relaxation (Figure 3a). In contrast, incubation with a higher concentration of hydrogen peroxide (1 mM) for 15 min followed by washout led to potentiation of acetylcholine-induced relaxation (Figure 3b). However, this potentiation was not sustained since when tested 2 h later, maximal acetylcholine (3 μM)-induced relaxation was reduced from $91.1 \pm 3.2\%$ to $27.4 \pm 4.5\%$, $n=9$. If incubation in the presence of hydrogen peroxide (1 mM) was extended to 30 or 60 min followed by washout, subsequent acetylcholine-induced relaxation was powerfully inhibited (Figure 3b). Following pretreatment with catalase (1000 u ml^{-1}) the effects of hydrogen peroxide (1 mM) on acetylcholine-induced relaxation were completely prevented (Figure 4). In contrast, catalase (1000 u ml^{-1}) failed to reverse the blockade of acetylcholine-induced relaxation seen following treatment with hydrogen peroxide (1 mM) for 60 min (maximal acetylcholine-induced relaxation $39.3 \pm 5.1\%$ and $35.6 \pm 4.9\%$, $n=5$, in the absence and presence of catalase, respectively).

Effects of 3-amino–1,2,4-triazole on the ability of hydrogen peroxide to impair acetylcholine-induced relaxation

Treatment of endothelium-containing rings with 3-amino-1,2,4-triazole (50 mM) for 90 min followed by washout had no effect by itself on acetylcholine (10 nM – 3 μM)-induced relaxation (Figure 5a). Furthermore, 3-amino-1,2,4-triazole did not influence the inability of hydrogen peroxide (0.1 mM, present during the final 30 min of the 90 min incubation followed by washout) to affect subsequent acetylcholine-induced relaxation. In contrast, treatment with 3-amino-1,2,4-triazole led to a profound enhancement of the ability of hydrogen peroxide (1 mM, 30 min) to impair subsequent acetylcholine-induced relaxation (Figure 5b).

Effects of hydrogen peroxide and 3-amino-1,2,4-triazole on the endothelium-independent relaxation induced by glyceryl trinitrate and isoprenaline

In endothelium-denuded rings, glyceryl trinitrate (1 – 1000 nM) and isoprenaline (10 nM – 3 μM) induced concentration-dependent relaxation (Figure 5c – f). Incubation with hydrogen peroxide at 1 mM, but not 0.1 mM, for 30 min followed by washout led to depression of relaxation induced by glyceryl trinitrate or isoprenaline. Incubation with 3-amino-1,2,4-triazole (50 mM) for 90 min followed by washout had no effect by itself on subsequent relaxation to glyceryl trinitrate or isoprenaline and had no influence on the inability of hydrogen peroxide (0.1 mM, present during the final 30 min of the 90 min incubation) to affect relaxation to these agents. However, 3-amino-1,2,4-triazole did profoundly enhance the ability of hydrogen peroxide (1 mM) to depress relaxation to glyceryl trinitrate or isoprenaline (Figure 5d and f).

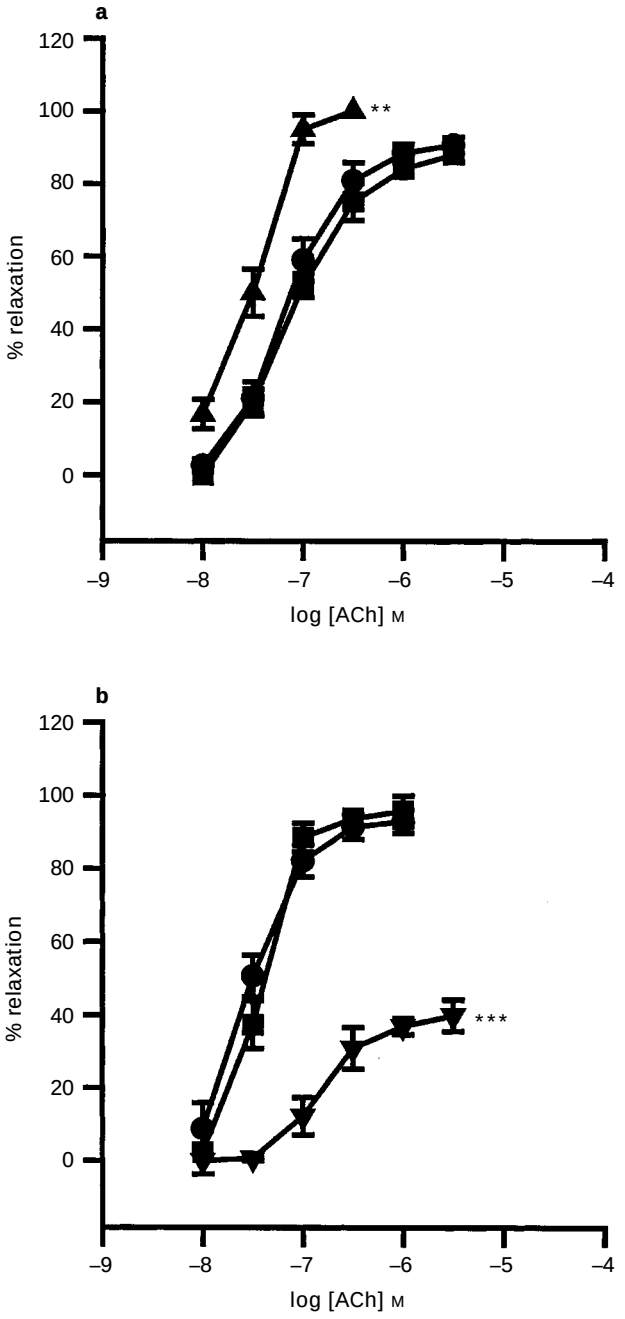

Figure 4 Concentration-response curves showing relaxation to acetylcholine (ACh, ●) on phenylephrine-contracted endothelium-containing rings of rat aorta, potentiation of this relaxation after 15 min incubation with hydrogen peroxide (H_2O_2, 1 mM) followed by washout (a, ▲), and blockade of relaxation after 60 min incubation with H_2O_2 (1 mM) followed by washout (b, ▼). Pretreatment with catalase (1000 u ml^{-1}, ■) completely protected against both the augmentation (a) and inhibition (b) produced by H_2O_2. Each point is the mean and vertical lines show s.e.mean of 5 – 6 observations. $^{**}P<0.005$ and $^{***}P<0.001$ indicate a significant difference from maximal relaxations obtained in untreated rings.

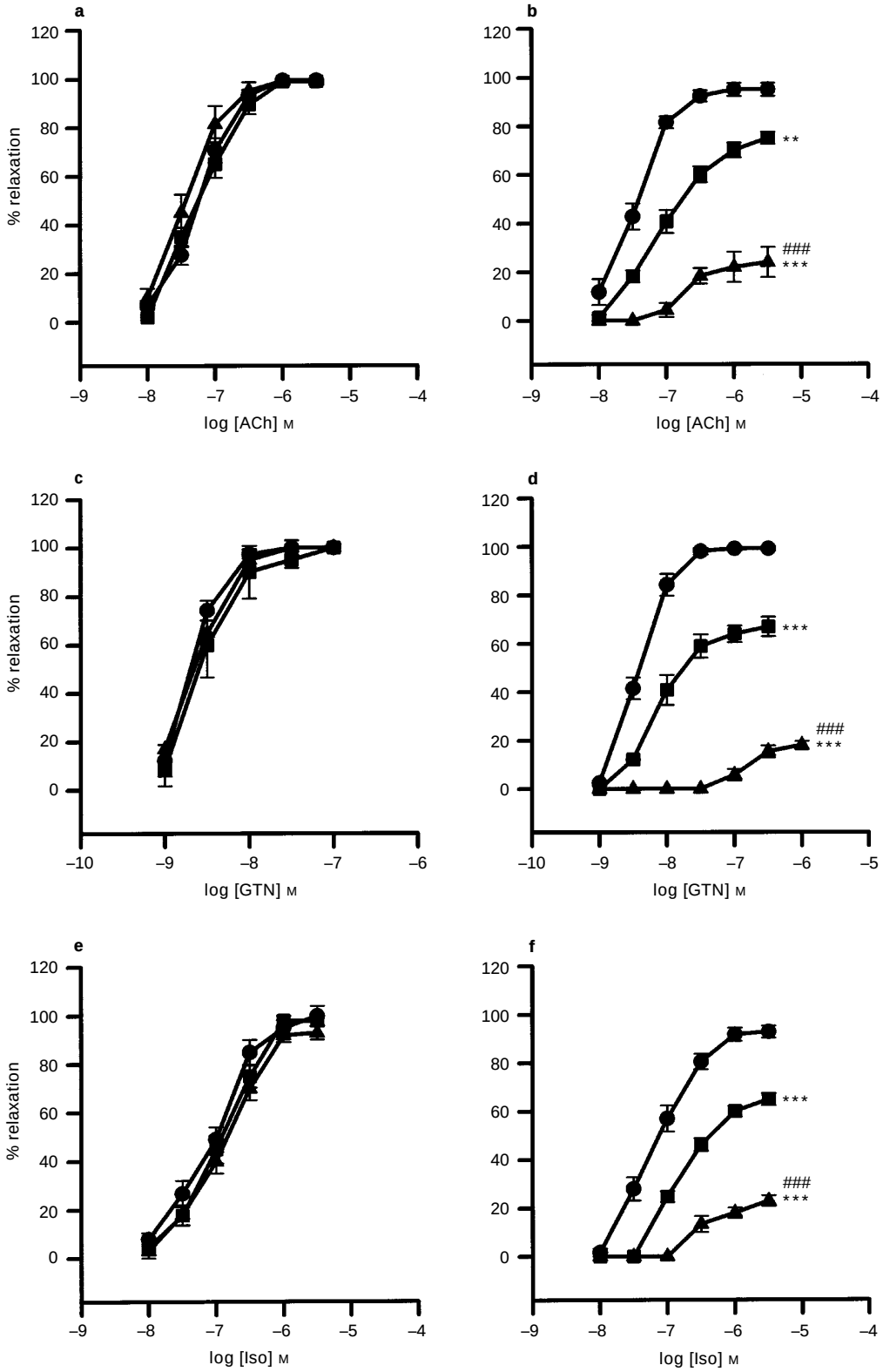

Figure 5 (a, c and e) Concentration-response curves (●) showing relaxation to (a) acetylcholine (ACh, 10 nM – 3 μM) on endothelium-containing rings of rat aorta and (c) glyceryl trinitrate (GTN, 1 – 100 nM) and (e) isoprenaline (Iso, 10 nM – 3 μM) on endothelium-denuded rings, and the lack of effect of incubation with hydrogen peroxide (H_2O_2, 0.1 mM, 30 min, followed by washout), either alone (■) or together with 3-amino-1,2,4-triazole (50 mM, 90 min, ▲) on these relaxations. (b, d and f) Concentration-response curves (●) showing relaxation to (b) acetylcholine (ACh, 10 nM – 3 μM), (d) glyceryl trinitrate (GTN, 1 – 100 nM) and (f) isoprenaline (Iso, 10 nM – 3 μM) and blockade of relaxation following incubation with H_2O_2 (1 mM, 30 min, followed by washout, ■). Furthermore, following treatment wtih 3-amino-1,2,4-triazole (50 mM, 90 min), incubation with H_2O_2 (present for the final 30 min of the 90 min incubation, followed by washout) now produced a higher degree of blockade (▲) of relaxation induced by all three vasodilators. Each point is the mean and vertical lines show s.e.mean of 6 – 10 observations. $^{**}P<0.005$ and $^{***}P<0.001$, indicate a significant difference from maximal relaxation in untreated rings. $^{###}P<0.001$ indicates a significantly greater blockade following combined treatment with 3-amino-1,2,4-triazole and H_2O_2 than with H_2O_2 alone.

Discussion

In this study we investigated the impairment of vascular responsiveness induced by hydrogen peroxide in rat aorta and explored the role of endogenous catalase in protection against this impairment.

We found that hydrogen peroxide produced profound relaxation of endothelium-containing and endothelium-denuded rings of rat aorta. Such relaxation results both directly from activation of smooth muscle soluble guanylate cyclase by hydrogen peroxide and indirectly through release of nitric oxide in endothelium-containing preparations (Burke & Wolin, 1987; Furchgott, 1991; Mian & Martin, 1995). When hydrogen peroxide at 1 mM but not 0.1 mM was present for up to 60 min followed by washout, the ability of the tissues to contract subsequently to phenylephrine was severely depressed in a time-dependent manner. This action, in contrast to the acute relaxant action of hydrogen peroxide, was not reversed by treatment with catalase and was essentially irreversible. Previous studies have demonstrated that this depression did not result from oxidation of the contractile agent, phenylephrine, but reflected impairment of smooth muscle function (Wolin & Belloni, 1985; Iesaki *et al*., 1994).

The effects of incubation with hydrogen peroxide were also investigated on the endothelium-dependent relaxation of rat aorta induced by acetylcholine. Incubation with hydrogen peroxide at 1 mM but not 0.1 mM, followed by washout, did produce powerful effects. Specifically, incubation with hydrogen peroxide (1 mM) for 15 min led to an unexpected potentiation of relaxation at each concentration of acetylcholine. However, this potentiation was not sustained, since a second concentration-response curve to acetylcholine constructed after 2 h on the same tissues revealed substantial blockade of relaxation. Thus, the initial potentiation appeared to be a prelude to more permanent blockade by hydrogen peroxide. In contrast to the short exposure, incubation with hydrogen peroxide (1 mM) for 30 or 60 min led to acetylcholine-induced relaxation being greatly inhibited. As expected, pretreatment with catalase (1000 u ml^{-1}) completely prevented the ability of hydrogen peroxide to increase initially and then impair acetylcholine-induced relaxation. In contrast, once blockade was established it could not be reversed either by washing or by subsequent treatment with catalase. These findings thus demonstrate an overall concentration- and time-dependent, and essentially irreversible impairment of endothelium-dependent relaxation by hydrogen peroxide.

In order to determine if the depression of acetylcholine-induced, endothelium-dependent relaxation resulted from damage to the endothelium or vascular smooth muscle, experiments were conducted with two relaxants which act in an endothelium-independent manner, namely glyceryl trinitrate and isoprenaline. We found that pretreatment with hydrogen peroxide at 1 mM, but not 0.1 mM, for 30 min also depressed the relaxant effects of these two agents, suggestive of damage to the smooth muscle. Additional endothelial damage to endothelium-containing vessels is also likely to have occurred, since previous results from our laboratory have demonstrated impairment of barrier function of endothelial monolayers by hydrogen peroxide (Berman & Martin, 1993).

An explanation for the damaging actions of hydrogen peroxide may lie in its actions on intracellular Ca^{2+} homeostasis. It has been shown that exposure of vascular endothelial cells (Hirosumi *et al*., 1988; Geeraerts *et al*., 1991) and vascular smooth muscle cells (Krippeit-Drews *et al*., 1995), as well as cardiac myocytes (Josephson *et al*., 1991) and renal tubular cells (Ueda & Shah, 1992) to hydrogen peroxide leads to sustained increases in intracellular Ca^{2+} levels. Although enhanced activity of calcium-dependent nitric oxide synthase may explain the early augmentation of acetylcholine-induced relaxation seen in this study, the final outcome of a sustained elevation of intracellular calcium is tissue injury via lipid peroxidation (Hirosumi *et al*., 1988; Geeraerts *et al*., 1991), activation of Ca^{2+}-dependent proteases (Geeraerts *et al*., 1991), and alterations in the cytoskeleton (Hinshaw *et al*., 1989).

The potential role of endogenous catalase in protecting vascular reactivity against hydrogen peroxide-induced damage was investigated with the use of 3-amino-1,2,4-triazole (Heim *et al*., 1956; Margoliash & Novogrodsky, 1958). In these experiments, treatment of rings with 3-amino-1,2,4-triazole (50 mM) alone had no effect on subsequent contraction to phenylephrine or relaxation to acetylcholine, glyceryl trinitrate or isoprenaline. Thus, 3-amino-1,2,4-triazole produced no non-selective effects, in keeping with our previous findings (Mian & Martin, 1995). Furthermore, treatment with 3-amino-1,2,4-triazole had no effect on the inability of hydrogen peroxide at 0.1 mM to influence the actions of these vasoactive agents. In contrast, this treatment with 3-amino-1,2,4-triazole potentiated the ability of hydrogen peroxide at 1 mM to depress responses to each of these agents. These findings indeed suggest an important role for endogenous catalase in protecting vascular smooth muscle cells from damage induced by high (1 mM) but not lower (0.1 mM) concentrations of hydrogen peroxide.

The concentrations of hydrogen peroxide found *in vivo* are difficult to predict, but resting concentrations as high as 0.025 mM have been found in mammalian tissues (Halliwell & Gutteridge, 1989). Furthermore, higher concentrations are known to be encountered following inhibition of scavenging enzymes and in the vicinity of activated leucocytes (Suttorp *et al*., 1986). It is important to note that although inhibition of endogenous catalase with 3-amino-1,2,4-triazole potentiated vascular impairment induced by 1 mM hydrogen peroxide, it had no effect on the actions of a lower concentration (0.1 mM) of this oxidant. Consistent with these findings are previous studies demonstrating in many cell types that catalase acts as a high capacity, but low affinity scavenger of hydrogen peroxide, and is more biologically relevant at high concentrations of the oxidant (Cohen & Hochstein, 1963; Nicholls, 1972; Jones *et al*., 1981; Dobrina Patriarca, 1986; Verkerk & Jondkind, 1992). In contrast, glutathione peroxidase acts as a high affinity, but low capacity scavenger, preferentially metabolizing low concentrations of hydrogen peroxide. The possibility that inhibition of this enzyme might potentiate damage by low concentrations of hydrogen peroxide is worthy of consideration but beyond the scope of the present investigation.

In conclusion, our findings suggest that the oxidant, hydrogen peroxide, induces a non-selective impairment of vascular smooth muscle function in rat aorta, resulting in loss of contractile and relaxant actions. Potentiation of these actions by 3-amino-1,2,4-triazole is consistent with a role for endogenous catalase in protecting vascular smooth muscle function from oxidant damage by hydrogen peroxide. The possibility that a component of the impairment of acetylcholine-induced relaxation by hydrogen peroxide involved an additional damaging action to the endothelium is likely.

K.B.M. is supported by a University of Glasgow Postgraduate Scholarship. We are grateful to the B.H.F. for financial support.

References

BERMAN, R.S. & MARTIN, W. (1993). Arterial endothelial dysfunction: actions of homocysteine and the hypoxanthine-xanthine oxidase free radical generating system. *Br. J. Pharmacol.*, **108,** 920–926.

BURKE, T.M. & WOLIN, M.S. (1987). Hydrogen peroxide elicits pulmonary arterial relaxation and guanylate cyclase activation. *Am. J. Physiol.*, **252,** H721–H732.

CHANCE, B. (1950). The reactions of catalase in the presence of the notatin system. *Biochem. J.*, **46,** 384–402.

CHEESEMAN, K.H. & SLATER, T.F. (1993). An introduction to free radical biochemistry. In *Free Radicals in Medicine*. ed. Cheeseman, K.H. & Slater, T.F. pp. 481–493. London: Churchill Livingston.

COHEN, G. & HOCHSTEIN, P. (1963). Glutathione peroxidase: the primary agent for the elimination of hydrogen peroxide in erythrocytes. *Biochemistry*, **2,** 1420–1428.

DIONISI, O., GALEOTTI, T., TERRANOVA, T. & AZZI, A. (1975). Superoxide radicals and hydrogen peroxide formation in mitochondria from normal and neoplastic tissues. *Biochem. Biophys. Acta*, **403,** 292–300.

DOBRINA, A. & PATRIARCA, P. (1986). Neutrophil-endoethelial cell interaction. Evidence for and mechanisms of the self-protection of bovine microvascular endothelial cells from hydrogen peroxide-induced oxidative stress. *J. Clin. Invest.*, **78,** 462–471.

FREEMAN, B.A. & CRAPO, J.D. (1982). Biology of disease: free radicals and tissue injury. *Lab. Invest.*, **47,** 412–426.

FURCHGOTT, R.F. (1991). Interactions of H_2O_2 and NO in modifying tone of vascular smooth muscle: the SOD paradox. In *Resistance Arteries, Structure and Function*. ed. Mulvany, M.J. pp. 216–220. New York: Elsevier Science Publishers B.V.

GEERAERTS, M.D., RONVEAUX-DUPAL, M.F., LEMASTERS, J.L. & HERMAN, B. (1991). Cytosolic free Ca^{2+} and proteolysis in lethal oxidative injury in endothelial cells. *Am. J. Physiol.*, **261,** C889–C896.

HALLIWELL, B. (1982). Production of superoxide, hydrogen peroxide and hydroxyl radicals by phagocytic cells: a cause of chronic inflammatory disease? *Cell Biology International Reports*, **6,** 529–542.

HALLIWELL, B. & GUTTERIDGE, J.M.C. (1989). *Free Radicals in Biology and Medicine*. Second Edition. p. 80. Oxford: Clarendon Press.

HARLAND, J.M. & CALLAHAN, K.S. (1984). Role of hydrogen peroxide in the neutrophil-mediated release of prostacyclin from cultured endothelial cells. *J. Clin. Invest.*, **74,** 442–448.

HEIM, W.G., APPLEMAN, D. & PYFROM, H.T. (1956). Effects of 3-amino-1,2,4-triazole on catalase and other compounds. *Am. J. Physiol.*, **186,** 19–23.

HINSHAW, D.B., BURGER, J.M., ARMSTRONG, B.C. & HYSLOP, P.A. (1989). Mechanism of endothelial cell shape change in oxidant injury. *J. Surg. Res.*, **46,** 339–349.

HIROSUMI, J., OUCHI, Y., WATANABE, M., KUSUNOKI, J., NAKAMURA, T. & ORIMO, H. (1988). Effect of superoxide and lipid peroxide on cytosolic free calcium concentration in cultured pig aortic endothelial cells. *Biochem. Biophys. Res. Commun.*, **152,** 301–307.

IESAKI, T., OKADA, T., YAMAGUCHI, H. & OCHI, R. (1994). Inhibition of vasoactive amine induced contractions of vascular smooth muscle by hydrogen peroxide in rabbit aorta. *Cardiovasc. Res.*, **28,** 963–968.

JONES, D.P., EKLÖW, L., THOR, H. & ORRENIUS, S. (1981). Metabolism of hydrogen peroxide in isolated hepatocytes: relative contributions of catalase and glutathione peroxidase in decomposition of endogenously generated H_2O_2. *Arch. Biochem. Biophys.*, **210,** 505–516.

JOSEPHSON, R.A., SILVERMAN, H.S., LAKATTA, E.G., STERN, M.D. & ZWEIER, J.L. (1991). Study of the mechanisms of hydrogen peroxide and hydroxyl free radical-induced cellular injury and calcium overload in cardiac myocytes. *J. Biol. Chem.*, **266,** 2354–2361.

KATSUKI, S., ARNOLD, W.P., MITTAL, C.K. & MURAD, F. (1977). Stimulation of guanylate cyclase by sodium nitroprusside, nitroglycerine and nitric oxide in various tissue preparations and comparison to the effects of sodium azide and hydroxylamine. *J. Cyclic Nucleotide Res.*, **3,** 23–35.

KRIPPEIT-DREWS, P., HABERLAND, C., FINGERLE, J., DREWS, G. & LANG, F. (1995). Effects of H_2O_2 on membrane potential and $[Ca^{2+}]i$ of cultured rat arterial smooth muscle cells. *Biochem. Biophys. Res. Commun.*, **209,** 139–145.

LEWIS, M.S., WHATLEY, R.E., CAIN, P., MCINTYRE, T.M., PRESCOTT, S.M. & ZIMMERMAN, G.A. (1988). Hydrogen peroxide stimulates the synthesis of platelet-activating factor by endothelium and induces endothelial cell-dependent neutrophil adhesion. *J. Clin. Invest.*, **82,** 2045–2055.

MARGOLIASH, E. & NOVOGRODSKY, A. (1958). A study of the inhibition of catalase by 3-amino-1:2:4-triazole. *Biochem. J.*, **68,** 468–475.

MIAN, K.B. & MARTIN, W. (1995). The inhibitory effect of 3-amino-1,2,4-triazole on relaxation induced by hydroxylamine and sodium azide but not hydrogen peroxide or glyceryl trinitrate in rat aorta. *Br. J. Pharmacol.*, **116,** 3302–3308.

MIAN, K.B. & MARTIN, W. (1996). Potentiation of hydrogen peroxide-induced impairment of vasodilator activity of rat aorta by 3-amino-1,2,4-triazole. *Br. J. Pharmacol.*, **119,** 304P.

MISRA, H.P. (1974). Generation of superoxide free radical during the autoxidation of thiols. *J. Biol. Chem.*, **249,** 2151–2155.

NICHOLLS, P. (1972). Contributions of catalase and glutathione peroxidase to red cell peroxide removal. *Biochem. Biophys. Acta*, **279,** 306–309.

SERVANIAN, A. & HOCHSTEIN, P. (1985). Mechanisms and consequences of lipid peroxidation in biological systems. *Ann. Rev. Nutr.*, **5,** 365–369.

SETTY, B.N.Y., JUREK, E., GANLEY, C. & STUART, M.J. (1984). Effects of hydrogen peroxide on vascular arachidonic acid metabolism. *Prostaglandins Leukotrienes Med.*, **14,** 205–213.

SUTTORP, N., TOEPFER, W. & ROKA, L. (1986). Antioxidant defense mechanisms of endothelial cells: glutathione redox cycle versus catalase. *Am. J. Physiol.*, **251,** C671–C680.

UEDA, N. & SHAH, S.V. (1992). Role of intracellular calcium in hydrogen peroxide-induced renal tubular cell injury. *Am. J. Physiol.*, **263,** F214–F221.

VERKERK, A. & JONDKIND, J.F. (1992). Vascular cells under peroxide stress: a balance study on in vitro peroxide handling by vascular endothelial and smooth muscle cells. *Free Radical. Res. Commun.*, **17,** 121–132.

WALDMAN, S.A. & MURAD, F. (1987). Cyclic GMP synthesis and function. *Pharmacol. Rev.*, **39,** 163–196.

WOLIN, M.S. & BELLONI, F.L. (1985). Superoxide anion selectively attenuates catecholamine-induced contractile tension in isolated rabbit aorta. *Am. J. Physiol.*, **249,** H1127–H1133.

(Received January 10, 1997
Revised March 10, 1997
Accepted March 17, 1997)