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INDUCTION OF VASCULAR RELAXATION BY HYDROPEROXIDES

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SUMMARY: Hydrogen peroxide, tert-butyl hydroperoxide, cumene hydroperoxide, and 3-chloroperoxybenzoic acid (CPB) and 15-HPETE relaxed, in a concentration dependent manner rat aortic rings contracted with $PGF_{2\alpha}$ ($1x10^{-5}$). Relaxation is not inhibited by either indomethacin ($2x10^{-5}$M), a cyclo-oxygenase inhibitor or eicosatetraynoic acid ($1x10^{-5}$M), a dual cyclo-oxygenase and lipoxygenase inhibitor. Rings with intact endothelium relaxed to a greater degree on exposure to CPB and 15-HPETE. Methylene blue, a soluble guanylate cyclase inhibitor ($1x10^{-5}$M) blocked the relaxation elicited by the five peroxides, whereas both superoxide dismutase (scavenger of superoxide anion) and mannitol (scavenger of hydroxyl radical) have no effect. We conclude that relaxation of vascular smooth muscle is a general property of peroxides and that the endothelium may in some instances facilitate this effect.

The existence of an endothelium derived vascular relaxant factor (EDRF) was first reported by Furchgott and Zawadski for acetylcholine induced relaxation of rabbit aorta (1). Since then, several other agents were shown to release EDRF in different blood vessels of different mamalian species (2-5). The identity of EDRF is unknown but there is general agreement that the vascular relaxation observed during endothelial-activation is associated with increased activity of soluble guanylate cyclase in the smooth muscle (6,7). Agents which increase the activity of soluble guanylate cyclase include free radicals, fatty acid peroxides, nitric oxide and hydrogen peroxide (8-10). Previous studies have shown that the effect of EDRF could be blocked or attenuated by anti-oxidants or agents which inhibit the release of fatty acids from membrane phospholipids (11,12). This raises the possibility that EDRF may be a free radical or a peroxide derived from a fatty acid. In the current investigation, we have tested several peroxides to evaluate their effect on smooth muscle relaxation in the presence and absence of endothelium.

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METHODS

Hydrogen peroxide, tert-butyl hydroperoxide, cumene hydroperoxide, indomethacin, mannitol and superoxide dismutase (SOD, bovine blood, 2800 U/mg) were purchased from Sigma. 3-chloroperoxy benzoic acid (CPB) was from Aldrich. Eicosatetraynoic acid (ETYA) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) were gifts from Roche and Upjohn respectively. Stock solutions of hydrogen peroxide and tert-butyl hydroperoxide were prepared in distilled deionized water and cumene hydroperoxide and CPB were prepared in 95% ethanol. The method described by Crawford et al (13) was used for the preparation of 15-hydroperoxy-eicosatetraenoic acid (15-HPETE).

Male Sprague Dawley rats, (200-300 gm) kept under standard lighting conditions and diet were decapitated and the thoracic aortae removed. Ring segments (3-4 mm) were prepared to avoid stripping of endothelium and mounted in 5 ml glass chambers. The chambers were filled with Krebs-bicarbonate buffer (pH 7.4; millimolar composition: NaCl, 116; KCl, 4.7; $MgSO_4$, 1.2; $CaCl_2$, 2.5; NaH_2PO_4, 1.2; $NaHCO_3$, 23; glucose, 11) equilibrated with 5% CO_2 in oxgen and warmed to 37°C. The vessels were maintained at 1gm tension for an hour. Tension was measured isometrically with a force transducer (Harvard). In certain vessel preparations, the endothelium was disrupted by rubbing a metal probe against the intimal surface. The efficacy of the endothelium removal was tested using acetylcholine as suggested by Furchgott and Zawadski (1). The different hydroperoxides were added to the bath in cumulative doses after the rings were pre-contracted with $PGF_{2\alpha}$ ($1x10^{-5}M$). In experiments using inhibitors of arachidonic acid metabolism, the reagents were added 15 min. prior to the addition of $PGF_{2\alpha}$.

RESULTS

The vessels were contracted with $PGF_{2\alpha}$ ($1x10^{-5}M$), and the five peroxides, hydrogen peroxide, cumene hydroperoxide, tert-butyl hydroperoxide, CPB and 15-HPETE were found to relax rat aortic rings in a concentration dependent manner (10^{-6}-$10^{-3}M$) (Figs 1 and 2). The three peroxides, hydrogen peroxide, tert-butyl hydroperoxide and cumene hydroperoxide relaxed vascular rings with and without endothelium to the same degree which is in contrast to the situation observed with acetylcholine where relaxation is dependent on the endothelium. On the otherhand, CPB, exhibited a slight endothelial dependency (Figs. 2-A and B) in that rings with intact endothelium relaxed at lower doses ($2x10^{-6}M$) when compared with vessels without endothelium. In addition, intact vessels were contracted by higher doses of CPB ($2x10-^{5}M$); vessels without endothelium required a ten-fold higher dose of CPB ($1x10-^{4}M$) for contraction. The response to 15-HPETE is shown in figure 1-D. It is apparent that the vessels with endothelium relaxed significantly more compared to vessels without endothelium.

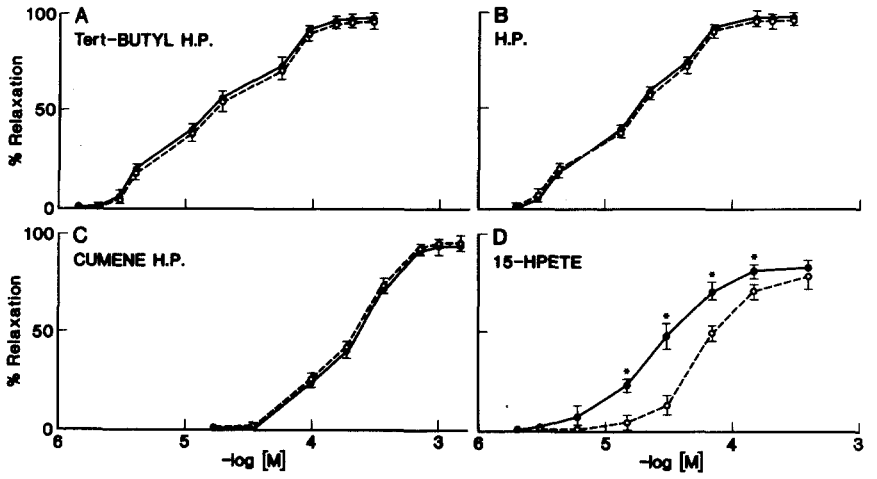
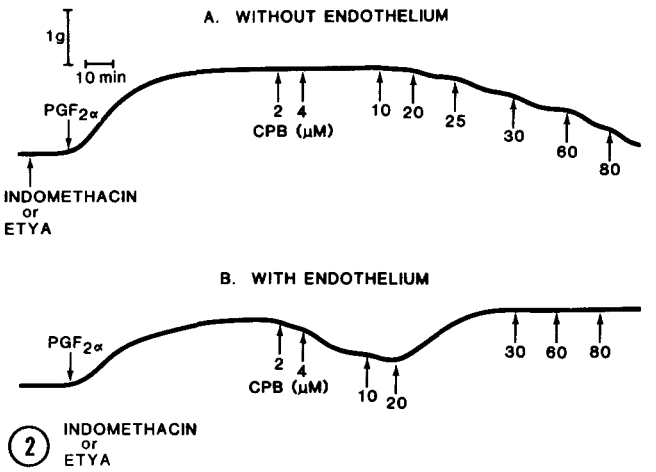


Figure I. Effect of Tert-Butyl H.P. (A), Hydrogen Peroxide (B), Cumene H.P. (C), and 15-HPETE (D) on rat aortic rings with (___) and without (- -) endothelium. Rings are contracted with $PGF_{2\alpha}$ ($1x10^{-5}$M). Results are mean ± S.E.M. of five separate experiments. H.P. stands for hydroperoxide. * $P< 0.05$.

To test the effect of various inhibitors of arachidonic acid metabolism on the response of aortic vessels to the peroxides, the vessels were incubated with indomethacin ($2x10^{-5}$M), a cyclo-oxygenase inhibitor and ETYA ($1x10^{-5}$M), a dual cyclo-oxygenase/lipoxygenase inhibitor, prior to the addition of $PGF_{2\alpha}$. Both indomethacin and ETYA had no effect on relaxation (Figs. I and 2) showing that the relaxation is not due to the generation of any cyclo-oxygenase or lipoxygenase products of arachidonic acid by the peroxides employed in our experiments.

EDRF is believed to induce relaxation of vascular muscle by activation of soluble guanylate cyclase (I4) and methylene blue is a known inhibitor of this enzyme (I5). Methylene blue ($1x10^{-5}$M) completely blocks the relaxation elicited by hydrogen peroxide and 15-HPETE (Figs. 3-A and B). A similar inhibition was observed for the other peroxides by methylene blue. This indicates that the mechanisms of vascular relaxation elicited by the peroxides may be due to the activation of soluble guanylate cyclase in smooth muscle.



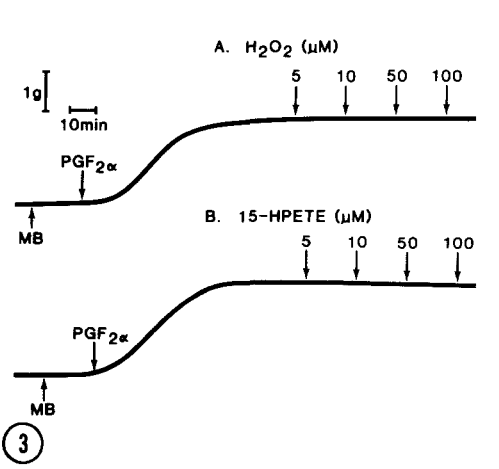


Figure 2. Effect of CPB on rat aortic rings without (A) and with endothelium B. Rings are contracted with $PGF_{2\alpha}$ (1×10^{-5}M).

Figure 3. Effect of methylene blue (MB) (1×10^{-5}M) on H_2O_2 (A) and 15-HPETE (B) elicited relaxation of rat aortic rings pre-contracted with $PGF_{2\alpha}$ (1×10^{-5}M)

Both superoxide anion and hydroxyl radicals could be generated from the peroxides. In order to test whether peroxide mediated relaxation is due to the generation of these radicals, vessels were incubated with mannitol (10 mM), a hydroxyl radical scavenger and superoxide dismutase (10 ug/ml), a superoxide scavenger. However, both of the scavengers had no appreciable inhibitory effect (data not shown).

DISCUSSION

Intense efforts have been directed in the past few years to identify EDRF and to elucidate its mechanism of action (2-7). Several agents have been suggested as possible candidates for EDRF (16). Our studies show that various peroxides can relax vascular rings in a concentration dependent manner. The relaxation is not inhibited by either indomethacin or ETYA suggesting that relaxation is not mediated by release of either cyclo-oxygenase or lipoxygenase products.

There were differences in the nature of relaxation elicited by these peroxides. Hydrogen peroxide, cumene hydroperoxide and tert-butyl hydroperoxide elicited relaxation which was independent of the endothelium

(Figs. 1-A, B&C). On the otherhand both 15-HPETE and CPB relaxed vessels with endothelium to a greater extent than those without endothelium (Figs. 1-D & 2-B respectively). In fact CPB exhibited contractile properties at higher doses ($2x10^{-5}M$), a property which was not shared by any of the other hydroperoxides. In addition, except in the case of CPB, acetylcholine relaxed vascular rings to the same degree before and after exposure to the peroxides (data not shown). Thus the concentration of the peroxides used and the short duration of exposure of the aortic rings do not damage the functional integrity of the endothelium, except in the case of CPB.

Endothelial cells can synthesize 15-(s)-hydroxy-eicosatetraenoic acid (15-HETE) and 8,15-(s) - dihdroxy-eicosatetraenoic acid (8,15-diHETE) from the precursor 15-HPETE (17). But recent studies (11,18) have shown that both 15-HETE and 8,15-diHETE do not possess any vascular relaxing properties. in contrast, 15-HPETE is reported to relax cerebral arterioles (19) and canine femoral artery (12). Prostacyclin is the major metabolite of arachidonic acid produced by the endothelial cells (20) but the relaxation produced by the peroxides cannot be due to prostacyclin since the experiments were done in presence of indomethacin. Moreover, peroxides are known to inhibit prostacyclin synthetase (21).

Vasodilation by EDRF is associated with an increase in the activity of soluble guanylate cyclase in smooth muscle (7). Our experiments with methylene blue, an inhibitor of soluble guanylate cyclase (15) show that methylene blue blocks the relaxation elicited by all five peroxides. Thus the peroxides may elicit relaxation through activation of soluble guanylate cyclase in smooth muscle which is in accord with previous reports (8-10) that hydrogen peroxide and other fatty acid peroxides activate soluble guanylate cyclase.

Since the peroxides described here can generate various radicals, including superoxide anion and hydroxyl radical, we tested the effect of SOD (scavenger of superoxide anion) and mannitol (scavenger of hydroxyl

radical) on vascular relaxation elicited by the various peroxides. Both SOD and mannitol have no inhibitory effect on vascular relaxation (data not shown). This shows that vascular relaxation elicited by the peroxides are not mediated through the generation of hydroxyl radical or superoxide anion. This is in agreement with a recent report (22) on the effect of hydroxyl radical and superoxide anion on endothelium-mediated relaxation of rabbit aorta. Interestingly, another recent study (23) showed that acetylcholine-induced relaxation is moderately depressed using mannitol suggesting that hydroxyl radical is involved in triggering release of EDRF.

In conclusion, our experiments show that various peroxides elicit vascular relaxation, which may be due to direct activation of soluble guanylate cyclase in smooth muscle and that the relaxation is not mediated through the generation of any cyclo-oxygenase or lipoxygenase products or through hydroxyl radical or superoxide anion generation. The nature of peroxide-elicited relaxation using various fatty acid hydroperoxides is a promising approach to deducing the identity of EDRF.

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