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The vasodilatation induced by hydroperoxy metabolites of arachidonic acid in the rat mesenteric and pulmonary circulation

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1 The effects of 15-hydroperoxy metabolites of arachidonic acid on vascular tone were evaluated in the perfused mesenteric preparation, the isolated perfused lung and segments of pulmonary arteries of the rat.

2 In the mesenteric preparation, precontracted with phenylephrine, both 15-hydroperoxy-5,8,11,13-eicosatetraenoic acid (15-HPETE, ED_{50} 1.6 nmol) and 8,15-dihydroperoxy-5,9,11,13-eicosatetraenoic acid (8,15-diHPETE, ED_{50} 0.3 nmol) induced dose-dependent vasodilatation, whereas 5,15-diHPETE (0.2–100 nmol) had no effect. Prostacyclin (ED_{50} 0.01 nmol) was, however, more potent than the hydroperoxides.

3 In the rat isolated lung, precontracted with the stable thromboxane agonist U-46619, dose-dependent decrease in the perfusion pressure occurred with 15-HPETE (ED_{50} 40 nmol), 5,15-diHPETE (ED_{50} 30 nmol) and 8,15-diHPETE (ED_{50} 7 nmol) while 13-hydroperoxide of linoleic acid had no effect. Prostacyclin was 10 times more potent than 8,15-diHPETE. The vasodilator effects were not affected by indomethacin.

4 In both endothelium intact and denuded rat pulmonary arteries the hydroperoxides 15-HPETE, 8,15-diHPETE, and 5,15-diPETE induced dose-dependent relaxation. The hydroperoxide, 8,15-diHPETE was at least 3 times more potent than 15-HPETE or 5,15-diHPETE. The hydroperoxides had no effect on the basal tone of vessel segments and the relaxation induced by 15-HPETE was not attenuated by methylene blue (5 μM).

5 These data indicate that 8,15-diHPETE may be a significant endothelium-independent vasodilator product of arachidonate lipoxygenation.

Introduction

The prominent lipoxygenase in both the vascular endothelium (Hopkins *et al.*, 1984) and the lung (Hamberg *et al.*, 1980) is 15-lipoxygenase. Arachidonic acid is metabolized in human endothelial cells by 15-lipoxygenase to metabolites, such as 15-hydroperoxy-5,8,11,13-eicosatetraenoic acid (15-HPETE), 15-hydroxy-5,8,11,13-eicosatetraenoic acid (15-HETE), 8-15-dihydroperoxides (8,15-diHPETEs) and corresponding dihydroxy acids (8,15-diHETEs) (Hopkins *et al.*, 1984). In contrast, linoleic acid is metabolized by 15-lipoxygenase to 13-hydroperoxy-9,11-octadecadienoic acid (13-HPODE) and 13-hydroxy-9,11-octadecadienoic acid (13-HODE), which are continuously released from human vascular endothelium (Buchanan *et al.*, 1985). The predominant product of the cyclo-oxygenase pathway of arachidonic acid in endothelial cells is prostacyclin (Bunting *et al.*, 1976).

Endothelial eicosanoids from both the cyclo-oxygenase and 15-lipoxygenase pathways induce changes in the vascular tone of blood vessels; for example prostacyclin causes relaxation of the bovine coronary artery and contraction of the rat abdominal aorta (Dusting *et al.*, 1977; Van Dam *et al.*, 1986). The hydroperoxides induce vasodilatation of feline pial vessels, contraction of the coronary artery but have no effect on noradrenaline-precontracted rabbit aorta (Koide *et al.*, 1982; Christman *et al.*, 1984; Försterman

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& Neufang, 1984). One explanation of the variation between these findings is that the vascular effects of endothelium-derived eicosanoids are modified locally.

The purpose of these studies was to compare the actions of 15-HPETE, 8,15-diHPETE, 5,15-diHPETE, 13-HPODE and prostacyclin in selected isolated blood vessels and perfused vascular beds of male rats.

Methods

Animals

Mature male Sprague-Dawley rats (weighing 270–410 g) from Charles River Breeding Laboratories (Wilmington, MA) were the source of all organ and tissue preparations. The animals were exsanguinated under anaesthesia (sodium pentobarbitone, 40 mg kg^{-1}, i.p.).

The isolated perfused mesenteric vascular bed

A ventral midline incision was made and the mesentery was exteriorized into a Petri dish containing Krebs Ringer bicarbonate (KRB) of the following composition (mM): NaCl 118.2, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 25, glucose 5.6 and indomethacin 5.5 μM. The KRB solution was equilibrated with 95% O_2 and 5% CO_2 and kept at a constant temperature (37°C) and pH (7.4) throughout the experiment. The superior mesenteric artery was perfused as described by McGregor (1965) with the following modification to preserve the microvasculature: the mesenteric vascular bed was isolated with the intestine and perfused *in situ*. In preliminary studies complete dose-response curves for phenylephrine were obtained. From these data the dose of phenylephrine inducing 80% of the maximum response was determined to be 2 μM. After an equilibration period of 30 min, the KRB containing 2 μM phenylephrine was infused at 2.4 ml min^{-1}. When the pressure was stable acetylcholine (0.8 μM), was injected. Only those preparations were used in which acetylcholine induced more than 50% decrease in the pressure and only one dose-response curve was derived for the hydroperoxides on each preparation.

The isolated lung preparation

After heparinization and thoracotomy the main pulmonary artery was cannulated and the lungs were excised and transferred to a perfusion apparatus and rodent respirator as described by Heikelä *et al.* (1982). The isolated lungs were perfused through the pulmonary artery with 37°C KRB in a nonrecirculating system at a constant flow rate of 8 ml min^{-1}. The perfusion medium was aerated with 5% CO_2 in O_2. The pulmonary artery pressure was measured with a Harvard pressure transducer. After a 5 min equilibration period either U-46619 or prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) were infused at a flow rate of 0.1 ml min^{-1} (final concentration 25 and 200 nM, respectively). When a sustained increase in the perfusion pressure was obtained the different hydroperoxides or prostacyclin were injected into the main pulmonary artery. The effects of hydroperoxides and prostacyclin on the basal vascular tone were also evaluated. Experiments were performed in the presence and absence of indomethacin (10 μM) in the perfusate.

The isolated pulmonary artery preparation

Ring segments of the rat pulmonary artery were prepared essentially as described earlier by Cunard *et al.* (1986). The vessel segments were pre-contracted with submaximal concentrations of U-46619 (28 nM). The functional integrity of the endothelium in intact and denuded vessel segments was determined by using acetylcholine to test relaxation as described by Furchgott (1983). After contracting the segments with U-46619, dose-response relaxation curves were obtained for 15-HPETE (0.2 to 20 μM), 5,15-diHPETE (0.05 to 5 μM), 8,15-diHPETE (0.01 to 5 μM), and 13-HPODE (0.2 to 20 μM). The effect of the hydroperoxides was also evaluated in vessel segments precontracted with phenylephrine, histamine, noradrenaline, 5-hydroxytryptamine (5-HT) and $PGF_{2\alpha}$. Relaxation responses to different hydroperoxides were separated by periods of 60 to 90 min during which time the vessel segments were washed with 150 ml of the KRB solution. Indomethacin (10 μM) was always present in the KRB bathing solution.

Drugs

15(S)-hydroperoxy-5(Z),8(Z),11(Z),13(E)-eicosatetraenoic acid (15-HPETE) and the dihydroperoxides, 8(S),15(S)-dihydroperoxy-5(Z),9(E),11(Z),13(E)-eicosatetraenoic acid (8,15-diHPETE) and 5(S),15(S)-dihydroperoxy-6(E), 8(Z),11(Z), 13(E)-eicosatetraenoic acid (5,15-diHPETE) were prepared from arachidonic acid by soybean lipoxygenase and separated by h.p.l.c. (Baldwin *et al.*, 1978; Van Os *et al.*, 1981). 13(S)-hydroperoxy-9(Z),11(E)-octadecadienoic acid (13-HPODE) was prepared from linoleic acid by soybean lipoxygenase (Holman *et al.*, 1969). Soybean lipoxygenase, (−)-phenylephrine hydrochloride, indomethacin, noradrenaline, histamine, 5-HT, methylene blue and acetylcholine chloride were obtained from Sigma Chem. Co (St Louis, MO, U.S.A.). Prostacyclin (PGI_2), U-46619 (9,11-dideoxy-11α,9α-epoxymethano-prostaglandin $F_{2\alpha}$) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, tromethamine) were obtained from Upjohn Co (Kalamazoo, MI, U.S.A.).

Data analyses

The molar concentration of agonist which induced 50% of the maximum response (ED_{50}) was estimated graphically from the dose-response relaxation curves. All data are expressed as the mean ± s.e.mean.

Results

Rat perfused mesenteric vascular bed

The basal pressure of the perfused mesenteric vascular bed was 43.3 ± 2.7 mmHg ($n = 22$). Phenylephrine infusion (2 μM) increased the pressure by a further 54.6 ± 6 mmHg ($n = 22$). Bolus injections of 15-HPETE or 8,15-diHPETE (0.05 to 3.0 nmol) induced dose-dependent decreases in the perfusion pressure but the dihydroperoxide 5,15 diHPETE (0.2 to 100 nmol) was ineffective ($n = 4$) (Figures 1 and 2). The estimated ED_{50} values for 8,15-diHPETE and 15-HPETE were 0.3 and 1.6 nmol, respectively. Indomethacin had no effect on the relaxation induced by the hydroperoxides. Bolus injections of prostacyclin (1.3 to 42 pmol) decreased the perfusion pressure in the mesenteric vascular bed in the presence of indomethacin in a dose-dependent manner (Figure 1).

Rat perfused pulmonary bed

The mean perfusion pressure in the rat isolated lung was 8.8 ± 0.2 mmHg ($n = 57$). The thromboxane agonist U-46619 (25 nM) increased the pressure by a further 7.2 ± 0.2 mmHg ($n = 39$). Bolus injections of the hydroperoxides 15-HPETE, 5,15-diHPETE and 8,15-diHPETE induced dose-dependent decreases in the pulmonary artery pressure whereas 13-HPODE (2 to 200 nmol) had no effect (Figures 2 and 3). The estimated ED_{50} values for 8,15-diHPETE, 5,15-diHPETE, and 15-HPETE were 7, 30, and 40 nmol, respectively. The perfusion pressure decreased 10–15 s following the injection of 8,15-diHPETE and 20 to 30 s following the 15-HPETE and 5,15-diHPETE (Figure 2). The perfusion pressure returned to the preinjection levels 1 min after 8,15-diHPETE (1 to 2 nmol). Indomethacin (10 μM) did not affect the relaxation induced by the hydroperoxides. Prostacyclin was about ten times more potent than 8,15-diHPETE and the estimated ED_{50} valued for PGI_2 was 0.7 nmol. The vasodilator effects of 15-HPETE and 8,15-diHPETE were also evaluated after pulmonary vasoconstriction had been induced by $PGF_{2\alpha}$. Both, 15-HPETE and 8,15-diHPETE, induced dose-dependent vasodilatation to the same degree as observed during U-46619 infusion with approximate ED_{50} values of 50 nM and 7 nM, respectively. In contrast to the effects on preparations with increased vascular tone, none of the hydroperoxides had any effect on the basal perfusion pressure when 10 nmol 8,15-diHPETE or 5,15-diHPETE and 50 nmol 15-HPETE or 13-HPODE were injected into the pulmonary circulation. Prostacyclin (2 nmol) decreased the basal perfusion pressure by less than 0.5 mmHg (data not shown).

Pulmonary artery relaxation

The hydroperoxides 15-HPETE, 5,15-diHPETE, and 8,15-diHPETE elicited dose-dependent relaxations of U-46619 pre-contracted pulmonary artery rings which were intact (Figure 4a) or where the endothelium had

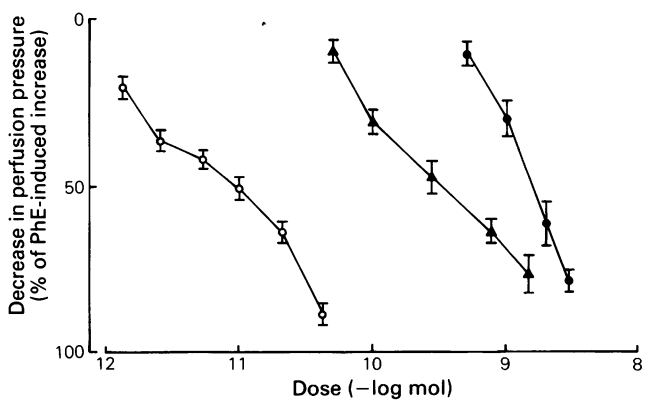

Figure 1 The effect of arachidonate hydroperoxides and prostacyclin on the perfusion pressure in the rat mesenteric vascular bed. The decreases in the pressure (induced with constant infusion of 2 μM phenylephrine) to bolus injections of 8,15-diHPETE (▲), 15-HPETE (●) and PGI_2 (○) are shown as a mean from at least 4 experiments with s.e.mean shown by vertical lines.

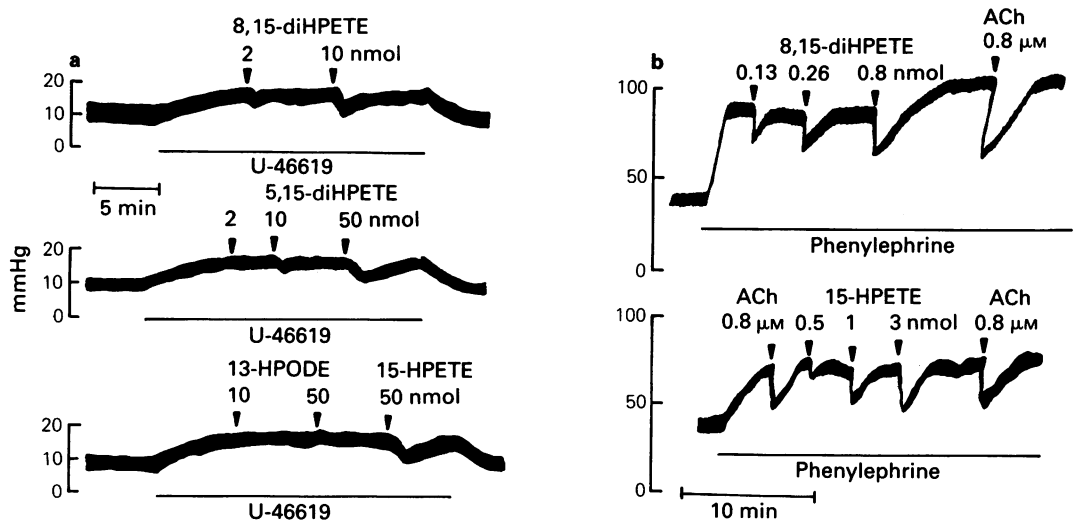

Figure 2 Representative recordings illustrating the effects of hydroperoxides on the pulmonary artery pressure of the rat isolated lung (a) and the perfusion pressure of the rat mesenteric vascular bed (b); The pressure in the pulmonary and mesenteric circulation was increased with infusion of 25 nM U-46619 and 2 μM phenylephrine, respectively. The vasodilator response (> 50% decreases) to 0.8 μM acetylcholine (ACh) was established for each mesenteric preparation.

been removed (Figure 4b). The tension induced by 28 μM U-46619 was 1.14 ± 0.04 g ($n = 21$) and 1.30 ± 0.12 g in intact and denuded ($n = 7$) vessel segments, respectively. The maximum relaxation induced by 13-HPODE (20 μM) in intact vessel segments ($n = 4$) was 20.9 ± 7.7%. The potency of the hydroperoxide 8,15-diHPETE was 3 fold that of 15-HPETE and 5 fold that of 5,15-diHPETE in the dose-range of 0.2 to 5 μM. In denuded vessels the potency of 8,15-diHPETE was 4 fold that of 15-HPETE over a dose-range of 0.2 to 1 μM. The relaxation induced by 13-HPODE was neither dose-dependent nor reproducible in denuded vessel segments ($n = 4$). The relaxant effects of 15-HPETE (5 μM) on intact pulmonary artery segments ($n = 5$) were the same whether the segments were contracted with $PGF_{2\alpha}$ or U-46619. In contrast, regardless of the dose, 15-HPETE had little or no effect on intact or denuded pulmonary artery segments contracted with histamine, 5-HT, noradrenaline, or phenylephrine. In intact vessel segments ($n = 3$), the relaxation induced by 15-HPETE was not affected by concentrations of methylene blue (5 μM, 15 min preincubation) which attenuated acetylcholine-induced relaxation by 82.7 ± 7.9%. The hydroperoxides 15-HPETE (5 μM) and 8,15-diHPETE (1 μM) did not affect basal tension of intact vessel segments ($n = 2$) at doses that produced marked relaxation when the same vessels were contracted with U-46619. The relaxation induced by 15-HPETE (5 μM) and 8,15-diHPETE (1 μM) in intact vessel segments was stable for up to 1 h but the relaxation induced by lower concentrations began to decay within 5 min.

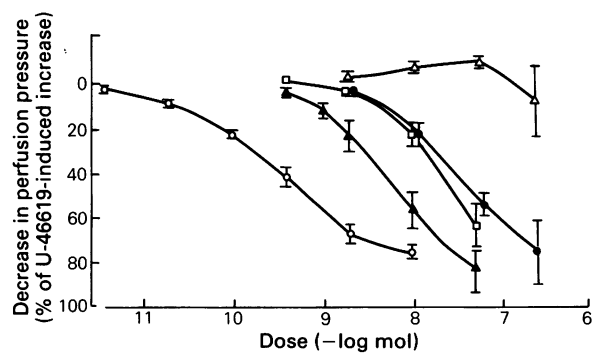

Figure 3 The dose-dependent decrease in the pressure induced by bolus injections of hydroperoxides into the pulmonary circulation during U-46619 (25 nM) infusion. The responses to 15-HPETE (●), 5,15-diHPETE (□), 8,15-diHPETE (▲), 13-HPODE (△) and prostacyclin (○) are expressed as a mean for at least 4 animals; s.e.mean shown by vertical lines.

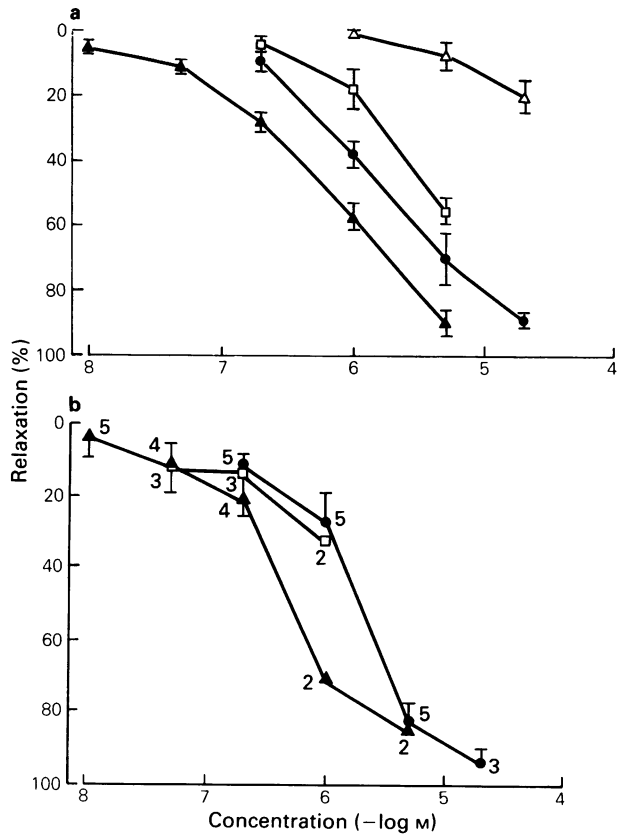

Figure 4 Relaxant effects of hydroperoxides on rat isolated pulmonary artery ring segments with intact endothelium (a) or with endothelium removed (b). The rings were precontracted with U-46619 (28 nM). Symbols indicate: 8,15-diHPETE (▲), 15-HPETE (●), 5,15-diHPETE (□) and 13-HPODE (△). The results are shown as mean from at least 4 (a) or the indicated number of experiments (b); vertical lines show s.e.mean.

Discussion

The putative vasodilator properties of lipoxygenase products are of considerable interest in the light of recent studies on endothelial lipoxygenation of arachidonate and linoleate (Hopkins *et al.*, 1984; Buchanan *et al.*, 1985). Here we describe the properties of three hydroperoxides of arachidonate and one of linoleate. The most important finding was that 8,15-diHPETE is a potent and reversible vasodilator in the rat perfused mesenteric preparation which was constricted with phenylephrine. It was about ten times less potent than prostacyclin. The estimated ED_{50} values for PGI_2, 8,15-diHPETE and 15-HPETE were: 0.01, 0.3 and 1.6 nmol, respectively. Neither 5,15-diHPETE nor 13-HPODE was effective.

In the perfused lung preparation 8,15-diHPETE was the most potent compound (ED_{50} 7 nmol) followed by 5,15-diHPETE (ED_{50} 30 nmol) and 15-HPETE (ED_{50} 40 nmol). Again 13-HPODE was ineffective. Prostacyclin was ten fold (ED_{50} 0.7 nmol) more potent than 8,15-diHPETE. Unlike PGI_2 the hydroperoxides did not affect the basal perfusion pressure. Indomethacin did not modify the response to the hydroperoxides. Consequently it is unlikely that vasodilatation was due to the generation of prostacyclin.

All the hydroperoxides relaxed to some degree the pulmonary artery preparations which were precontracted with U-46619. The potency rank order was similar to that obtained with the perfused lung preparation in that 8,15-diHPETE and 13-HPODE were the most and least active compounds respectively.

Methylene blue reduced the acetycholine-induced endothelium-dependent relaxation but had no such effect on the relaxation response to hydroperoxides.

These data and the lack of effect of removing the endothelium indicate that the relaxant effect of the hydroperoxides is due to a direct action on the vascular smooth muscle. This is supported by the recent finding of Herman *et al.* (1986) that 15-HPETE relaxes a variety of arteries precontracted with $PGF_{2\alpha}$ and that the endothelium was not necessary for this effect.

Because both $PGF_{2\alpha}$ and U-46619 possess a hydroxyl group at carbon-15, one may speculate that the effect of 15-hydroperoxides is due to the receptor interaction with $PGF_{2\alpha}$ and U-46619. This possibility was excluded in experiments with mesenteric vascular bed contracted with phenylephrine. Because both 15-HPETE and 8, 15-diHPETE elicited a vasodilatation also in this preparation, this study indicates that these 15-hydroperoxides of arachidonate (and/or their metabolite) may have a vasodilator role.

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References

BALDWIN, J.E., DAVIES, D.I., HUGHES, L. & GUTTERIDGE, N.J.A. (1978). Synthesis from arachidonic acid of potential prostaglandin precursors. *J. Chem. Soc.* Perkin I, 115–121.

BUCHANAN, M.R., HAAS, T.A., LAGARDE, M. & GUICHARDANT, M. (1985). 13-Hydroxyoctadecadienoic acid is the vessel wall chemorepellant factor, LOX. *J. biol. Chem.*, **260,** 16056–16059.

BUNTING, S., GRYGLEWSKI, R., MONCADA, S. & VANE, J.R. (1976). Arterial walls generate from prostaglandin endoperoxides a substance (prostaglandin X) which relaxes strips of mesenteric and coeliac arteries and inhibits platelet aggregation. *Prostaglandins*, **12,** 897–913.

CHRISTMAN, C.W., WEI, E.P., KANTOS, H.A., POVLISHOCK, J.T. & ELLIS, E.F. (1984). Effects of 15-hydroperoxy-eicosatetraenoic acid (15-HPETE) on cerebral arterioles of cat. *Am. J. Physiol.*, **247,** H631–H637.

CUNARD, C.M., MADDOX, Y.T. & RAMWELL, P.W. (1986). Endothelium-dependent gender differences in the response of the rat pulmonary artery to the thromboxane mimic (U46619). *J. Pharmac. exp. Ther.*, **237,** 82–85.

DUSTING, G.J., MONCADA, S. & VANE, J.R. (1977). Prostacyclin (PGX) is the endogenous metabolite responsible for relaxation of coronary arteries induced by arachidonic acid. *Prostaglandins*, **13,** 3–15.

FURCHGOTT, R.F. (1983). Role of endothelium in responses of vascular smooth muscle. *Circulation Res.*, **53,** 557–573.

FÖRSTERMAN, U. & NEUFANG, B. (1984). The endothelium dependent relaxation of rabbit aorta: effects of antioxidants and hydroxylated eicosatetraenoic acids. *Br. J. Pharmac.*, **82,** 765–767.

HAMBERG, M., HEDQVIST, P. & RÅDEGRAN, K. (1980). Identification of 15-hydroxy-5,8,11,13-eicosatetraenoic acid (15-HETE) as a major metabolite of arachidonic acid in human lung. *Acta physiol. scand.*, **110,** 219–221.

HEIKELÄ, A., HAAVISTO, M., GRANNAS, R. & UOTILA, P. (1982). Pulmonary uptake of PGE_2 is inhibited by dipyridamole in rat isolated lungs. *Prostaglandins*, **23,** 147–156.

HERMAN, A.G., VAN DIEST, M. & VERBEUREN, T. (1986). Lipoxygenase metabolites of arachidonic acid cause contractions and relaxations of isolated canine blood vessels. In *Mechanisms of Vasodilatation.* ed. Vanhoutte, P.M. pp. 74–75. New York: Karger.

HOLMAN, R.T., EGWIM, P.O. & CHRISTIE, W.W. (1969). Substrate specificity of soybean lipoxidase. *J. biol. Chem.*, **244,** 1149–1151.

HOPKINS, N.K., OGLESBY, T.D., BUNDY, G.L. & GORMAN, R.R. (1984). Biosynthesis and metabolism of 15-hydroperoxy-5,8,11,13-eicosatetraenoic acid by human umbilical vein endothelial cells. *J. biol. Chem.*, **259,** 14048–14053.

KOIDE, T., NEICHI, T., TAKATO, M., MATSUSHITA, H., SUGIOKA, K., NAKANO, M. & HATA, S-I. (1982). Possible mechanisms of 15-hydroperoxyarachidonic acid-induced contraction of the canine basilar artery in vitro. *J. Pharmac. exp. Ther.*, **221,** 481–488.

McGREGOR, D.D. (1965). The effect of sympathetic nerve stimulation on vasoconstrictor responses in perfused mesenteric blood vessels of the rat. *J. Physiol.*, **177,** 21–30.

VAN DAM, J., MADDOX, Y.T., RAMWELL, P.W. & KOT, P.A. (1986). The role of the vascular endothelium in the contractile response to prostacyclin in the isolated rat aorta. *J Pharmac. exp. Ther.*, **239,** 390–394.

VAN OS, C.P.A., RIJKE-SCHILDER, G.P.M., VAN HALBEEK, H., VERHAGEN, J. & VLIEGENTHART, J.F.C. (1981). Double dioxygenation of arachidonic acid by soybean lipoxygenase I. Kinetics and regio-stereo specificities of the reaction steps. *Biochim. biophys. Acta*, **663,** 177–193.

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