ELSEVIER

European Journal of Pharmacology 378 (1999) 47–55

ejp

www.elsevier.nl/locate/ejphar

Influence of isoprostanes on vasoconstrictor effects of noradrenaline and angiotensin II

Wolfgang Sametz [a,*], Tanja Grobuschek [a], Susanne Hammer-Kogler [a], Heinz Juan [a], Reinhold Wintersteiger [b]

[a] *Department of Biomedical Research, University of Graz, Roseggerweg 48, Graz A-8036, Austria*
[b] *Department of Pharmaceutical Chemistry, University of Graz, Roseggerweg 48, Graz A-8036, Austria*

Received 15 February 1999; received in revised form 11 May 1999; accepted 15 June 1999

Abstract

The isoprostanes, 8-*iso*-prostaglandin $F_{2\alpha}$ and 8-*iso*-prostaglandin E_2, which are released in vivo by free radical-catalyzed peroxidation of arachidonic acid, are potent vasoconstrictors. Increased formation of 8-*iso*-prostaglandin $F_{2\alpha}$ has been detected in human cardiovascular diseases, in which enhanced plasma levels of noradrenaline and angiotensin II have harmful vasoconstrictor effects. Therefore, we investigated the influence of perfusions with the thromboxane A_2 mimetic, U 46619, and with the isoprostanes, 8-*iso*-prostaglandin $F_{2\alpha}$, 8-*iso*-prostaglandin E_2, 8-*iso*-prostaglandin E_1 and 8-*iso*-prostaglandin $F_{3\alpha}$, on the vasoconstrictor effects of noradrenaline and angiotensin II in the isolated perfused rabbit ear. Our results demonstrate that perfusions with U 46619, 8-*iso*-prostaglandin E_2 and 8-*iso*-prostaglandin $F_{2\alpha}$, at a subthreshold concentration (30 nM), amplified the vasoconstrictions induced by noradrenaline or angiotensin II significantly. In addition, the results show that U 46619, 8-*iso*-prostaglandin $F_{2\alpha}$, 8-*iso*-prostaglandin E_2 and 8-*iso*-prostaglandin E_1, which were applied as a bolus, induced much more pronounced vasoconstrictions than prostaglandin $F_{2\alpha}$, prostaglandin E_2 and prostaglandin $F_{3\alpha}$. Prostaglandin E_1 and 8-*iso*-prostaglandin $F_{3\alpha}$ showed no effects. In conclusion, it can be assumed that the powerful vasoconstrictions induced by 8-*iso*-prostaglandin E_2 and 8-*iso*-prostaglandin $F_{2\alpha}$ and their potentiating effects on vasoconstrictions induced by noradrenaline or angiotensin II might be of pathophysiological relevance in cardiovascular diseases.

Keywords: Isoprostane; Prostaglandin; U 46619; Noradrenaline; Angiotensin II; Vasoconstriction

1. Introduction

In recent years, it has been found that besides the traditional pathway of prostaglandin biosynthesis, an alternative, non-cyclooxygenase pathway leads to the formation of the so-called isoprostanes (Morrow et al., 1990a,b). These compounds, in which the two side chains are oriented *cis* to each other, are produced by free radical-catalyzed peroxidation of arachidonic acid in human and animals (Morrow et al., 1990a,b, 1994a, 1998a,b). Compared with prostaglandin $F_{2\alpha}$, which is a weak vasoconstrictor, and prostaglandin E_2, which is mainly a vasodilator (Nicosia and Patrono, 1989), the isoprostanes, 8-*iso*-prostaglandin $F_{2\alpha}$ and 8-*iso*-prostaglandin E_2, are powerful vasoconstrictors in vivo and in vitro (Morrow et al., 1994b; Kromer and Tippins, 1996; Möbert et al., 1997; Sametz et al., 1997, 1998).

Increased formation of 8-*iso*-prostaglandin $F_{2\alpha}$ was detected in patients with cardiovascular diseases (Davi et al., 1997; Delanty et al., 1997; Pratico et al., 1997; Mallat et al., 1998). Taking into consideration the important pathophysiological role of the endogenous vasoconstrictors, noradrenaline and angiotensin II, in cardiovascular diseases (Kopin, 1989; Schömig et al., 1991; Mancini, 1996; Riegger, 1996), the aim of this study was to investigate the influence of perfusions with the arachidonic acid metabolites, 8-*iso*-prostaglandin $F_{2\alpha}$, 8-*iso*-prostaglandin E_2, the dihomo-γ-linolenic acid metabolite, 8-*iso*-prostaglandin E_1, and the eicosapentaenoic acid metabolite, 8-*iso*-prostaglandin $F_{3\alpha}$, on the vasoconstrictor effects of noradrenaline and angiotensin II in the isolated perfused rabbit ear. This

* Corresponding author. Tel.: +43-316-385-3955; fax: +43-316-385-3956; E-mail: wolfgang.sametz@kfunigraz.ac.at

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00437-9

isolated organ is a model for peripheral vascular systems, which contains arteries, veins, arterioles, venules and capillary vessels (Burton, 1965). This in vitro model used has turned out to be useful for investigations of vascular effects of endogenous substances like noradrenaline, angiotensin II or neuropeptide Y (Juan and Sametz, 1986; Juan et al., 1988) and for determination of prostaglandin release induced by various compounds like bradykinin or histamine (Juan and Lembeck, 1976; Juan and Sametz, 1980).

2. Materials and methods

2.1. Materials

Purchased from Cayman Chemical (Ann Arbor, MI, USA) were 8-*iso*-prostaglandin $F_{2\alpha}$, 8-*iso*-prostaglandin E_2, 8-*iso*-prostaglandin E_1, 8-*iso*-prostaglandin $F_{3\alpha}$, 9,11-dideoxy-9,11-methanoepoxy prostaglandin $F_{2\alpha}$ (U 46619) and SQ 29548. On the other hand, noradrenaline and angiotensin II were from Sigma (Vienna, Austria). All drugs were dissolved and diluted in 0.9% saline freshly before experiments. The composition of Tyrode solution was (in mmol/l): NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.15, NaH_2PO_4 0.42, $NaHCO_3$ 11.9, glucose 5.6.

2.2. Isolated perfused rabbit ear

As described by Juan and Sametz (1986) in brief, rabbits of either sex (2.5–3.5 kg body weight; Zentrale Tierbiologische Einrichtung, Graz) were sacrificed by an overdose of pentobarbitone (> 50 mg/kg, i.v.). Isolated rabbit ears were perfused through their central artery with Tyrode solution (37°C, gassed with 95% O_2 and 5% CO_2). Flow was adjusted to 3 ml/min by constant pressure. Venous outflow was measured by an electronic drop recorder. A reduction of the venous outflow reflects vasoconstrictor effects.

2.3. Vascular actions of isoprostanes, prostaglandins and U 46619

Noradrenaline (bolus of 100 pmol) was given at intervals of 10 min as a testing substance until the reactivity of the vessels had become stable (at least after 60 min). Thereafter, dose–response curves (increasing single doses in moles) for 8-*iso*-prostaglandin $F_{2\alpha}$, 8-*iso*-prostaglandin E_2, 8-*iso*-prostaglandin E_1, 8-*iso*-prostaglandin $F_{3\alpha}$ or the thromboxane A_2 mimetic, U 46619, were established. For comparison, in each experiment, two of the agents were applied alternately at intervals of 5 min. In the first set of experiments, each isoprostane was compared with U 46619 and in a second, each isoprostane with its corresponding prostaglandin. Finally, for calculation (mean values), a number of 24 experiments were obtained for U 46619, 12 for isoprostanes and six for prostaglandins.

To see whether the sensitivity of the perfused organ to vascular actions of the substances used changes during the time course of the experiments, dose–response curves were repeated four times within a total period of 6 h. The vascular actions induced by these agents remained nearly unchanged with a maximum variation of $\pm 5\%$. To obtain comparable experimental conditions, the third dose–response curve of each substance from every experiment was used for calculation.

2.4. Influence of isoprostanes, prostaglandins and U 46619 on the vascular effects of noradrenaline and angiotensin II

In preliminary experiments, a subthreshold concentration of perfusions with isoprostanes, U 46619 and prostaglandins was determined, which caused effects on vasoconstrictions induced by noradrenaline or angiotensin II. Perfusions at a final concentration of 10 nM ($n = 4$) showed no effects on noradrenaline or angiotensin II induced vasoconstrictions, whereas perfusions with U 46619, 8-*iso*-prostaglandin $F_{2\alpha}$, 8-*iso*-prostaglandin E_2 and 8-*iso*-prostaglandin E_1, at a concentration of 100 nM ($n = 4$), caused weak vasoconstrictor responses by their own (data not shown).

Therefore, a final concentration of 30 nM was chosen, which was subthreshold for U 46619 and those isoprostanes. In the following experiments, the influence of perfusions with U 46619, 8-*iso*-prostaglandin $F_{2\alpha}$, 8-*iso*-prostaglandin E_2, 8-*iso*-prostaglandin E_1 and also with 8-*iso*-prostaglandin $F_{3\alpha}$, at this concentration on the vasoconstrictions induced by noradrenaline and angiotensin II, was investigated. Noradrenaline or angiotensin II was applied at intervals of 5 min as a bolus. For control, a dose–response curve of both vasoconstrictors was established before the start of perfusions. Thereafter, the dose–response curves were repeated during perfusions until the end of experiments ($n = 6$ for each experiment).

For comparison, the influence of perfusions with prostaglandin $F_{2\alpha}$, prostaglandin E_2, prostaglandin E_1 and prostaglandin $F_{3\alpha}$ at the same final concentration of 30 nM on the vasoconstriction induced by noradrenaline or angiotensin II was investigated in the same manner ($n = 6$ for each experiment). Mean values of noradrenaline and angiotensin II were calculated between 40 and 60 min after the start of perfusions.

2.5. Influence of 8-iso-prostaglandin $F_{2\alpha}$ on U 46619, of U 46619 on isoprostanes and of prostaglandin E_1 on U 46619 and isoprostanes

The influence of perfusions with 8-*iso*-prostaglandin $F_{2\alpha}$ (30 nM) on the vasoconstrictor effect of U 46619, of perfusions with prostaglandin E_1 (30 nM) on vasoconstric-

tions induced by U 46619, 8-*iso*-prostaglandin E_2, 8-*iso*-prostaglandin $F_{2\alpha}$ and 8-*iso*-prostaglandin E_1, and of perfusions with U 46619 (30 nM) on vasoconstrictions induced by isoprostanes, was investigated.

For control, dose–response curves of each vasoconstrictor (applied as a bolus) were established before the start of perfusions. Thereafter, the dose–response curves were repeated during the perfusions until the end of experiments ($n = 6$ for each experiment).

2.6. Influence of the thromboxane receptor antagonist, SQ 29548, on the interaction of 8-iso-prostaglandin E_2 with noradrenaline and 8-iso-prostaglandin $F_{2\alpha}$ with angiotensin II

After establishment of a dose–response curve of noradrenaline during perfusion with 8-*iso*-prostaglandin E_2 (30 nM), a perfusion with SQ 29548 (final concentration 10 nM) was started and continued together with the 8-*iso*-prostaglandin E_2 perfusion until the end of experiment ($n = 6$). The experiments with angiotensin II were performed in the same manner, but instead of 8-*iso*-prostaglandin E_2, 8-*iso*-prostaglandin $F_{2\alpha}$ was perfused together with SQ 29548.

2.7. Statistical analysis

Values are expressed as means ± S.E.M. Statistical significance was calculated by analysis of variance (ANOVA) followed by Dunnett's multiple comparison test with $P < 0.05$ being accepted as level of significance.

3. Results

3.1. Vascular actions of isoprostanes, U 46619 and prostaglandins

8-*Iso*-prostaglandin $F_{2\alpha}$, 8-*iso*-prostaglandin E_2,8-*iso*-prostaglandin E_1 and U 46619 induced vasoconstrictions in a dose-dependent manner in the vasculature of the isolated perfused rabbit ear (Fig. 1A).

In contrast, prostaglandin $F_{2\alpha}$, prostaglandin $F_{3\alpha}$ and prostaglandin E_2 showed only weak vasoconstrictions, whereas prostaglandin E_1 and 8-*iso*-prostaglandin $F_{3\alpha}$ had no effect (Fig. 1A,B). The following rank order of potency could be established: U 46619 > 8-*iso*-prostaglandin E_2 > 8-*iso*-prostaglandin $F_{2\alpha}$ > 8-*iso*-prostaglandin E_1 > prostaglandin $F_{2\alpha}$ > prostaglandin $F_{3\alpha}$ > prostaglandin E_2. ED_{50} values are summarized in Table 1.

3.2. Influence of perfusions with isoprostanes, U 46619 and prostaglandins on the vascular effects of noradrenaline and angiotensin II

Noradrenaline and angiotensin II induced vasoconstrictions in a dose-dependent manner in the vasculature of the isolated perfused rabbit ear. At subthreshold concentration

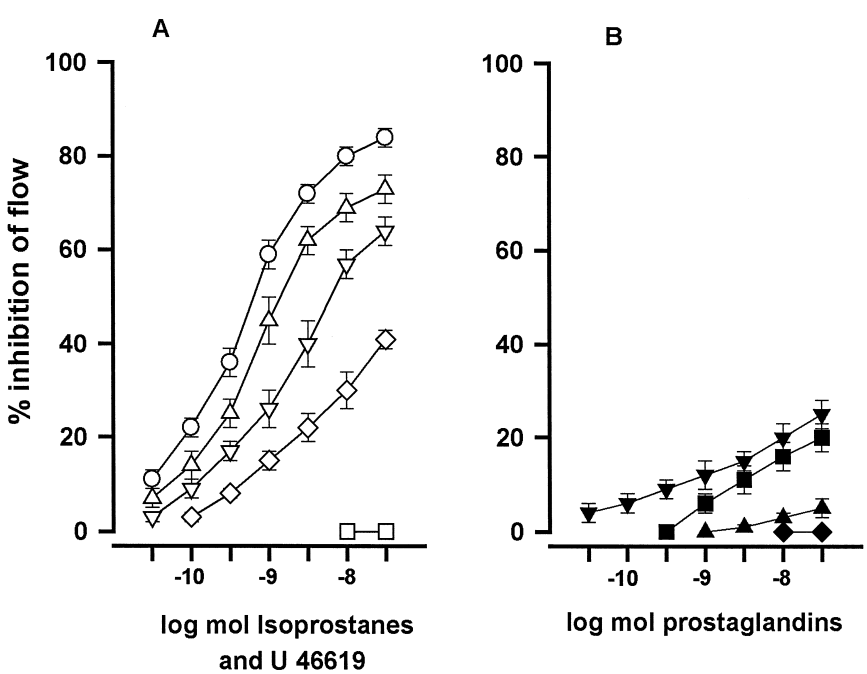

Fig. 1. Dose–response curves of vasoconstrictions (percent inhibition of flow) induced by bolus injections of (A) U 46619 (○), 8-*iso*-prostaglandin $F_{2\alpha}$ (▽), 8-*iso*-prostaglandin E_2 (△), 8-*iso*-prostaglandin E_1 (◇), 8-*iso*-prostaglandin $F_{3\alpha}$ (□) and of (B) prostaglandin $F_{2\alpha}$ (▼), prostaglandin E_2 (▲), prostaglandin E_1 (◆), prostaglandin $F_{3\alpha}$ (■). Data are means ± S.E.M.

Table 1
ED_{50} values of noradrenaline (NA), angiotensin II (AII), U 46619 and isoprostanes (8-*iso*-prostaglandin E_2, 8-*iso*-prostaglandin $F_{2\alpha}$, 8-*iso*-prostaglandin E_1) induced vasoconstrictions before (C) and during perfusions with isoprostanes (30 nM), U 46619 (30 nM) and prostaglandin E_1 (prostaglandin E_1; 30 nM) in the isolated perfused rabbit ear
For calculation of the ED_{50}, the dose–response curves were transformed to loglogit. Controls (C), not calculable (n.c.).

	ED_{50} (pmol)					
	NA	AII	U 46619	8-*Iso*-prostaglandin E_2	8-*Iso*-prostaglandin $F_{2\alpha}$	8-*Iso*-prostaglandin E_1
C	340	175	700	1400	7000	~ 70 000
+ U 46619	44	25	–	1550	7000	~ 70 000
+ 8-*Iso*-prostaglandin E_2	60	32	–	–	–	–
+ 8-*Iso*-prostaglandin $F_{2\alpha}$	75	50	690	–	–	–
+ 8-*Iso*-prostaglandin E_1	280	103	–	–	–	–
+ Prostaglandin E_1	4200	1900	14 000	22 000	~ 80|000	n.c.

(final concentration 30 nM), 8-*iso*-prostaglandin $F_{2\alpha}$, 8-*iso*-prostaglandin E_2 and U 46619 amplified significantly the vasoconstrictions induced by noradrenaline (Fig. 2A) or angiotensin II (Fig. 3A).

The maximum noradrenaline- or angiotensin II-enhancing effect induced by these isoprostanes or U 46619 was reached 20–30 min after the start of perfusions. Perfusions with 8-*iso*-prostaglandin E_1 at a final concentration of 30 nM showed a slight, but not significant, increasing effect on the vasoconstrictions induced by noradrenaline (Fig. 2A) or angiotensin II (Fig. 3A).

The ED_{50} of noradrenaline was reduced by U 46619 about 7.7-fold, by 8-*iso*-prostaglandin E_2 about 5.6-fold and by 8-*iso*-prostaglandin $F_{2\alpha}$ about 4.5-fold, that of angiotensin II about 7.0-, 5.5- and 3.5-fold, respectively. ED_{50} values are summarized in Table 1. Perfusions with prostaglandin $F_{2\alpha}$ or prostaglandin E_2 (final concentration 30 nM) did not influence the vasoconstriction induced by noradrenaline (Fig. 2B) or by angiotensin II (Fig. 3B). In contrast, perfusions with prostaglandin E_1 (final concentration 30 nM) reduced the vasoconstrictor effects of noradrenaline (Fig. 2B) as well as those of angiotensin II (Fig. 3B) significantly. The ED_{50} value of noradrenaline was enhanced by prostaglandin E_1 about 12-fold and that of angiotensin II about 11-fold. ED_{50} values are summarized in Table 1.

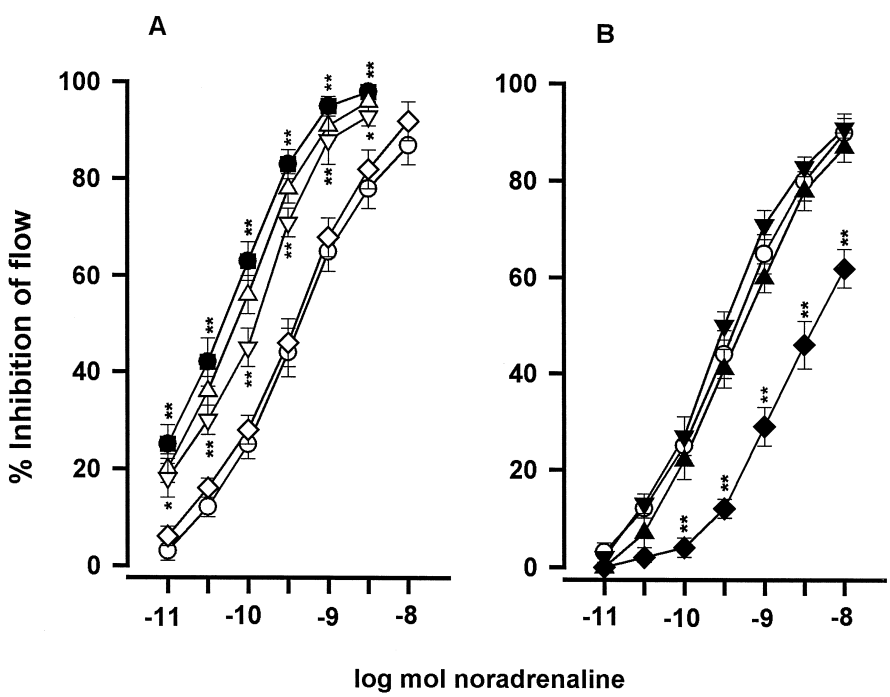

Fig. 2. Dose–response curves of vasoconstrictions (percent inhibition of flow) induced by bolus injections of noradrenaline before (○) and during perfusions (30 nM) with (A) U 46619 (●), 8-*iso*-prostaglandin $F_{2\alpha}$ (▽), 8-*iso*-prostaglandin E_2 (△), 8-*iso*-prostaglandin E_1 (◇) and with (B) prostaglandin $F_{2\alpha}$ (▼), prostaglandin E_2 (▲),prostaglandin E_1 (◆). Data are means ± S.E.M. Statistically significant differences: $^{*}P < 0.05$, $^{**}P < 0.001$.

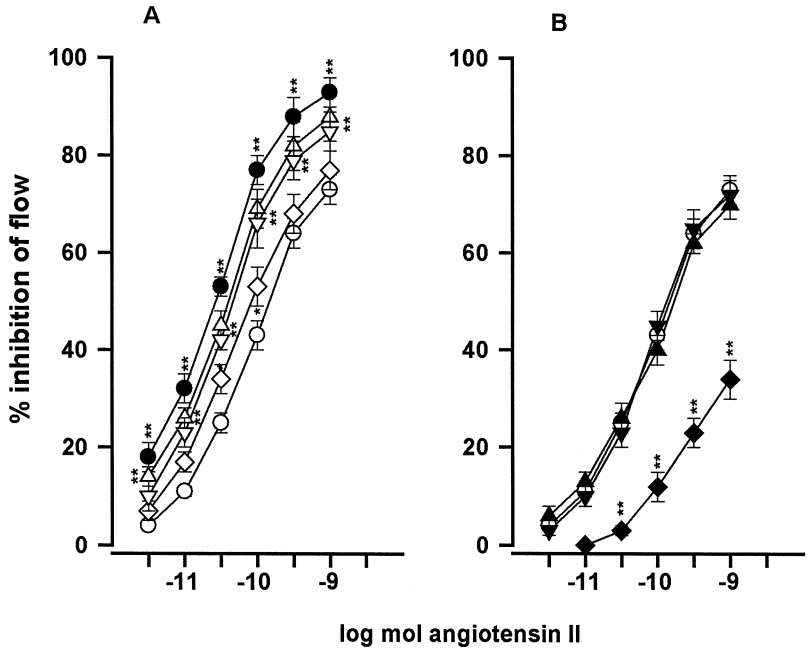

Fig. 3. Dose–response curves of vasoconstrictions (percent inhibition of flow) induced by bolus injections of angiotensin II before (○) and during perfusions (30 nM) with (A) U 46619 (●), 8-*iso*-prostaglandin $F_{2\alpha}$ (▽), 8-*iso*-prostaglandin E_2 (△), 8-*iso*-prostaglandin E_1 (◇) and with (B) prostaglandin $F_{2\alpha}$ (▼), prostaglandin E_2 (▲), prostaglandin E_1 (◆). Data are means ± S.E.M. Statistically significant differences: $^{*}P < 0.05$, $^{**}P < 0.001$.

Perfusions with 8-*iso*-prostaglandin $F_{3\alpha}$ or prostaglandin $F_{3\alpha}$ showed no influence on vasoconstrictions induced by noradrenaline or angiotensin II (data not shown).

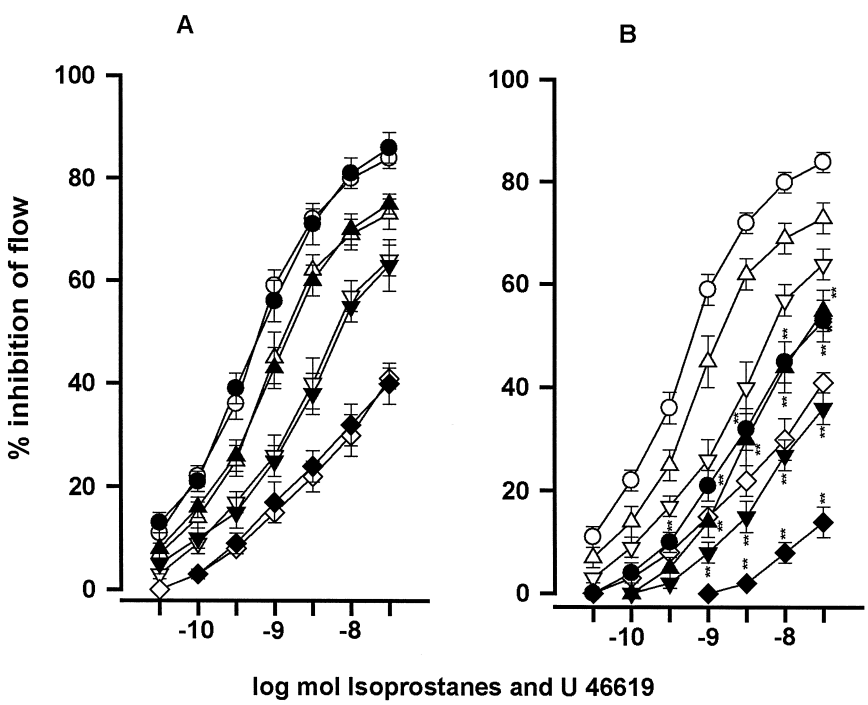

Fig. 4. Dose–response curves of vasoconstrictions (percent inhibition of flow) induced by bolus injections of (A) U 46619 before (○) and during perfusion (30 nM) with 8-*iso*-prostaglandin $F_{2\alpha}$ (●) and of 8-*iso*-prostaglandin $F_{2\alpha}$ (▽), 8-*iso*-prostaglandin E_2 (△), 8-*iso*-prostaglandin E_1 (◇) before and during perfusions (30 nM) with U 46619 (8-*iso*-prostaglandin $F_{2\alpha}$ ▼; 8-*iso*-prostaglandin E_2 ▲; 8-*iso*-prostaglandin E_1 ◆). Vasoconstrictions induced by bolus injections of (B) U 46419 (◦), 8-*iso*-prostaglandin $F_{2\alpha}$ (▽), 8-*iso*-prostaglandin E_2 (△), 8-*iso*-prostaglandin E_1 (◇) before and during perfusions (30 nM) with prostaglandin E_1 (U 46619 ●; 8-*iso*-prostaglandin $F_{2\alpha}$ ▼; 8-*iso*-prostaglandin E_2 ▲; 8-*iso*-prostaglandin E_1 ◆). Data are means ± S.E.M. Statistically significant differences: $^{**}P < 0.001$.

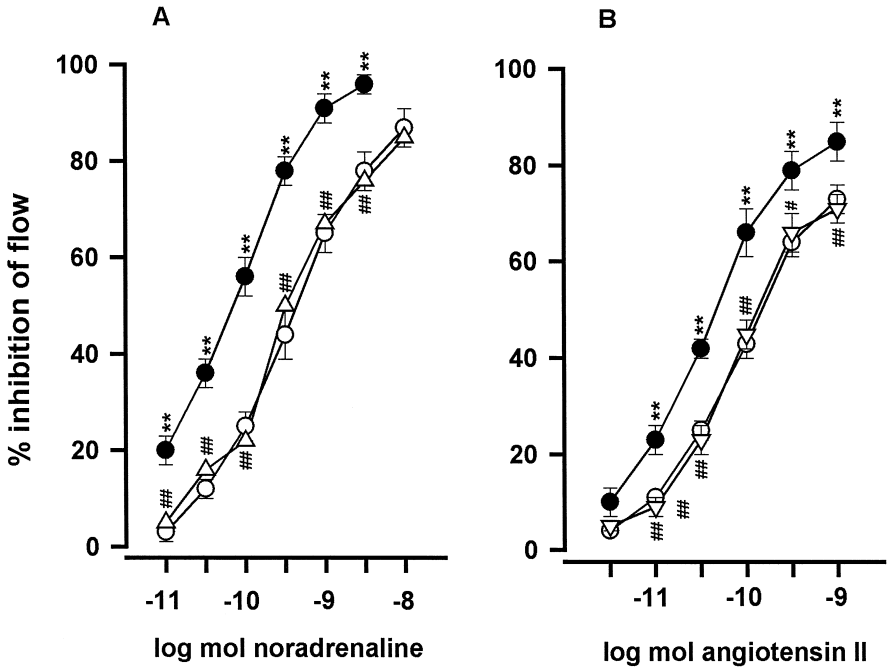

Fig. 5. Dose–response curves of vasoconstrictions (percent inhibition of flow) induced by bolus injections of (A) noradrenaline before (○), during a perfusion (30 nM) with 8-*iso*-prostaglandin E_2 (●) alone and together with a perfusion(10 nM) with SQ 29548 (△). Vasoconstrictions induced by bolus injections of (B) angiotensin II before (◦), during a perfusion (30 nM) with 8-*iso*-prostaglandin $F_{2\alpha}$ (●) alone and together with a perfusion(10 nM) with SQ 29548 (▽). Data are means ± S.E.M. Statistically significant differences: $^{**}P < 0.001$ noradrenaline or angiotensin II vs. noradrenaline + 8-*iso*-prostaglandin E_2 or angiotensin II + 8-*iso*-prostaglandin $F_{2\alpha}$. $^{\#}P < 0.05$, $^{\#\#}P < 0.001$ noradrenaline + 8-*iso*-prostaglandin E_2 or angiotensin II + 8-*iso*-prostaglandin $F_{2\alpha}$ vs. during SQ 29548.

3.3. Influence of 8-iso-prostaglandin $F_{2\alpha}$ on U 46619, of U 46619 on isoprostanes and of prostaglandin E_1 on U 46619 and isoprostanes

Perfusions with 8-*iso*-prostaglandin $F_{2\alpha}$ (30 nM) had no effect on vasoconstriction induced by U 46619 (Fig. 4A). Also, perfusions with U 46619 (30 nM) showed no enhancement of the vasoconstrictions induced by the isoprostanes used (Fig. 4A). Perfusions with prostaglandin E_1 (30 nM) reduced the vascular actions of U 46619 as well as of 8-*iso*-prostaglandin E_2, 8-*iso*-prostaglandin $F_{2\alpha}$ and of 8-*iso*-prostaglandin E_1 (Fig. 4B). The maximum inhibitory effect of prostaglandin E_1 was reached 10–20 min after the start of perfusion.

The ED_{50} values of U 46619, 8-*iso*-prostaglandin E_2 and 8-*iso*-prostaglandin $F_{2\alpha}$ were enhanced by prostaglandin E_1 about 20-, 16- and 11-fold, respectively. ED_{50} values are summarized in Table 1.

3.4. Influence of the thromboxane receptor antagonist, SQ 29548, on the interaction of 8-iso-prostaglandin E_2 with noradrenaline and 8-iso-prostaglandin $F_{2\alpha}$ with angiotensin II

We investigate the possible effects of the thromboxane receptor antagonist, SQ 29548, on the ability of 8-*iso*-prostaglandin E_2 to enhance vasoconstriction triggered by noradrenaline and the ability of 8-*iso*-prostaglandin $F_{2\alpha}$ to enhance vasoconstriction by angiotensin II. As soon as the amplifying effect of perfusions with those isoprostanes on noradrenaline or angiotensin II reached its maximum (20–30 min after start of perfusion), perfusions with SQ 29548 (final concentration 10 nM) were started.

Perfusion with 8-*iso*-prostaglandin E_2 (30 nM) amplified the vasoconstriction induced by noradrenaline significantly. This increased effect of noradrenaline was reduced during perfusion with SQ 29548 significantly to values similar to those observed before 8-*iso*-prostaglandin E_2 perfusion (Fig. 5A). Perfusion with 8-*iso*-prostaglandin $F_{2\alpha}$ (30 nM) amplified the vasoconstriction induced by angiotensin II significantly. This increasing effect on angiotensin II was also abolished by SQ 29548 (Fig. 5B). The maximum inhibitory effect of SQ 29548 was reached 40–60 min after the start of perfusion.

4. Discussion

It was found that 8-*iso*-prostaglandin $F_{2\alpha}$ and 8-*iso*-prostaglandin E_2 are formed in vivo by free radical-catalyzed peroxidation of arachidonic acid (C20: 4, $n-6$) (Morrow et al., 1990a,b, 1994a,b, 1998a,b). Consequently, isoprostanes derived not only from arachidonic acid but also from other polyunsaturated fatty acids could be formed by peroxidation in vivo. Indeed, it has been found that F-4-isoprostanes or F-3-isoprostanes were formed in vivo

and in vitro by radical-induced oxidation of the omega-3 fatty acids, docosahexaenoic acid (C22: 6, $n-3$) and eicosapentaenoic acid (C20: 5, $n-3$), respectively (Roberts et al., 1998; Nourooz-Zadeh et al., 1997). It can be assumed that also 8-*iso*-prostaglandin E_1 will be formed by peroxidation of dihomo-γ-linolenic acid (C20: 3, $n-6$).

Numerous experimental and clinical studies have suggested that eicosapentaenoic acid (for reviews, see Drevon et al., 1993; Schwandt, 1991) as well as dihomo-γ-linolenic acid (Kernoff et al., 1977; Szczeklik et al., 1984) have beneficial effects on the development of cardiovascular diseases. Free radicals play an important role in the occurrence and development of these diseases (Ross, 1986; Halliwell and Grootveld, 1987). Therefore, it is to be expected that besides cyclooxygenase products, 8-*iso*-prostaglandin $F_{3\alpha}$ and 8-iso prostaglandin E_1 will also be formed in patients on a diet enriched with eicosapentaenoic acid or dihomo-γ-linolenic acid. Taking these possibilities into consideration, it was of interest to investigate the vascular effects of both isoprostanes.

8-*Iso*-prostaglandin E_2 has been found to be a potent vasoconstrictor, approximately equipotent with that of 8-*iso*-prostaglandin $F_{2\alpha}$ (Morrow et al., 1994b; Sametz et al., 1997, 1998). This was an unexpected finding, because in most vascular systems, cyclooxygenase-derived prostaglandin E_2 is a vasodilator whereas prostaglandin $F_{2\alpha}$ is a vasoconstrictor, which has been attributed to the differences in ring structure and consequently, these prostaglandins act via different prostaglandin receptors (Andersen et al., 1976). Morrow and Roberts (1997) proposed that the stereochemistry of the side chains, rather than the ring structure, might be responsible for the vasoconstrictor action of both isoprostanes.

Our results show that 8-*iso*-prostaglandin E_2 at the ED_{50} is 50-fold more potent than 8-*iso*-prostaglandin E_1 in the vasculature of the rabbit ear. If we compare the effect of 8-*iso*-prostaglandin $F_{2\alpha}$ with that of 8-*iso*-prostaglandin $F_{3\alpha}$, we can see that the vasoconstriction induced by 8-*iso*-prostaglandin $F_{2\alpha}$ is in the range of 8-*iso*-prostaglandin E_2, whereas 8-*iso*-prostaglandin $F_{3\alpha}$ showed no vasoconstrictor effect. The lack of effect of 8-*iso*-prostaglandin $F_{3\alpha}$ was unexpected and surprising, because all other isoprostanes showed much more powerful vasoconstrictor effects than their corresponding prostaglandins. Thus, we must consider that the number of double bonds, in combination with the *cis*-orientation of the side chains, plays an important role in the vasoconstrictor effects of isoprostanes. To clarify this aspect, receptor binding studies are necessary.

In most diseases, e.g., atherosclerosis, in which free radicals play an important role, increased release of noradrenaline as well as angiotensin II occurs and isoprostanes are also enhanced (Kopin, 1989; Mancini, 1996; Pratico et al., 1997). Therefore, it was of interest to investigate the interaction between these endogenous vasoconstrictors.

Both isoprostanes, 8-*iso*-prostaglandin E_2 and 8-*iso*-prostaglandin $F_{2\alpha}$, and the thromboxane A_2 mimetic, U 46619, at subthreshold concentrations, amplified significantly vasoconstrictions induced by noradrenaline as well as angiotensin II in the vascular system of the rabbit ear. The present vasoconstrictor effects of noradrenaline and angiotensin II in this organ are in agreement with the results obtained by Juan and Sametz (1986). The effect of U 46619 is consistent with the results obtained by Stanton and Coupar (1988), who found that U 46619 amplified the vasoconstrictor responses of noradrenaline in the rat mesenteric vasculature in vitro. The authors inferred a pathophysiological role for thromboxane A_2 and noradrenaline when released together in vivo. The subthreshold concentration of the isoprostanes used in the present study were in the order of magnitude of those in rat plasma in association with free radical-catalyzed peroxidation induced by administration of CCl_4 or diquat (Morrow et al., 1990b) in vivo.

This increase of the vasoconstrictor action of noradrenaline or angiotensin II by these isoprostanes might be due to an overadditive effect (Pöch and Holzmann, 1980), which indicates that two compounds with the same effect (in our case, vasoconstriction) given together have two different points of attack (activation of different receptors).

In vascular smooth muscle cells, stimulation of thromboxane receptors as well as of α-adrenoceptors and angiotensin receptors result in an increase in intracellular free calcium, which leads finally to vasoconstrictions (for review, see Shaw and McGrath, 1996). Stanton and Coupar (1988) postulated the hypothesis that the amplifying effect of U 46619 on vasoconstrictions induced by noradrenaline is due to the ability of U 46619 to increase the utilization of intracellular calcium stores. We can speculate that this hypothesis is also relevant for the amplifying effects of the isoprostanes.

The prostaglandins, prostaglandin $F_{2\alpha}$, prostaglandin $F_{3\alpha}$ and prostaglandin E_2, as well as the isoprostane, 8-*iso*-prostaglandin $F_{3\alpha}$, at the concentration used did not influence the vasoconstriction induced by noradrenaline or angiotensin II. This might be due to the weak vasoconstrictor effects of these compounds.

The present results show that not only the vasoconstriction induced by U 46619 remained uninfluenced by subthreshold concentrations of 8-*iso*-prostaglandin $F_{2\alpha}$, but also the vasoconstrictor effect of the isoprostanes used by subthreshold concentrations of U 46619. This appears to indicate that isoprostanes act via the same receptor as the thromboxane A_2 mimetic, U 46619, in the vasculature of the rabbit ear. According to Ariëns et al. (1956), this phenomenon could be called a competitive synergism.

In the present study, the thromboxane receptor antagonist, SQ 29548, reduced the potentiating effect of isoprostanes on the vasoconstriction induced by noradrenaline or angiotensin II to control values. Taken together, all these results obtained in the vascular system of the rabbit

ear indicate that isoprostanes exert their vasoconstrictor effects via activation of the thromboxane receptor, which is in agreement with the assumption of Takahashi et al. (1992).

Prostaglandin E_1 has various desirable effects (for reviews, see Sinzinger and Rogatti, 1986; Diehm et al., 1991). The beneficial effects of prostaglandin E_1 or its precursor, dihomo-γ-linolenic acid, are utilized therapeutically in vascular diseases (Kernoff et al., 1977; Diehm et al., 1991). As our results show, prostaglandin E_1 inhibited vasoconstrictions induced by noradrenaline and angiotensin II as well as by 8-*iso*-prostaglandin $F_{2\alpha}$, 8-*iso*-prostaglandin E_2 and 8-*iso*-prostaglandin E_1. In contrast, 8-*iso*-prostaglandin E_1 amplified slightly the vasoconstrictor effects of noradrenaline and angiotensin II. This probable physiological antagonism of prostaglandin E_1, which is due to its vasodilator effect, on isoprostanes can be assessed as an additional beneficial effect and might be of importance in therapeutic use.

5. Conclusion

The results of the present study show that vasoconstrictions induced by the isoprostanes, 8-*iso*-prostaglandin $F_{2\alpha}$, 8-*iso*-prostaglandin E_2 and 8-*iso*-prostaglandin E_1, were much more pronounced than that of the corresponding prostaglandins in the vascular system of the isolated rabbit ear.

Reverse responses were obtained by the eicosapentaenoic acid metabolites — 8-*iso*-prostaglandin $F_{3\alpha}$ had no effect and prostaglandin $F_{3\alpha}$ caused similar weak vasoconstrictions as prostaglandin $F_{2\alpha}$. Thus, we can assume that the number of double bonds of the isoprostanes is important for their vasoactivity in the vasculature of the rabbit ear. The powerful vasoconstrictor actions of 8-*iso*-prostaglandin $F_{2\alpha}$ and 8-*iso*-prostaglandin E_2, together with their potentiating effects at subthreshold concentrations on vasoconstrictions induced by noradrenaline or angiotensin II, might be of pathophysiological relevance in cardiovascular diseases, in which free radicals play an important role.

References

Andersen, N.H., Ramwell, P.W., Leovey, E.M.K., Johnson, M., 1976. Biological consequences of prostaglandin molecular conformations. In: Samuelsson, B., Paoletti, R. (Eds.), Advances in Prostaglandin and Thromboxane Research, Vol. 1. Raven Press, New York, NY, pp. 271–289.

Ariëns, E.J., van Rossum, J.M., Simonis, A.M., 1956. A theoretical basis of molecular pharmacology: I. Interactions of one or two compounds with one receptor system. Arzneim.-Forsch. 6, 282–293.

Burton, A.C., 1965. Physiology and Biophysics of the Circulation. Year Book Medical Publisher, Chicago, IL.

Davi, G., Alessandrini, P.I., Mezzetti, A., Minotti, G., Bucciarelli, T., Costantini, F., Cipollone, F., Bon, G.B., Ciabattoni, G., Patrono, C., 1997. In vivo formation of 8-epi-prostaglandin F_2 alpha is increased in hypercholesterolemia. Arterial Thromboembolism Vasc. Biol. 17, 3230–3235.

Delanty, N., Reilly, M.P., Pratico, M.P., Lawson, J.A., McCarthy, J.F., Wood, A.E., Ohnishi, S.T., FitzGerald, D.J., FitzGerald, G.A., 1997. 8-Epi-PG F_2 alpha generation during coronary reperfusion. A potential quantitative marker of oxidant stress in vivo. Circulation 95, 2492–2499.

Diehm, C., Sinzinger, H., Rogatti, W., 1991. Prostaglandin E_1: New Aspects on Pharmacology, Metabolism and Clinical Efficacy. Springer-Verlag, Berlin.

Drevon, C.A., Baksaas, I., Krokan, H.E., 1993. Omega-3 Fatty Acids: Metabolism and Biological Effects. Birkhäuser Verlag, Basel.

Halliwell, B., Grootveld, M., 1987. The measurement of free radical reactions in humans. FEBS Lett. 213, 9–14.

Juan, H., Lembeck, F., 1976. Release of prostaglandins from the isolated perfused rabbit ear by bradykinin and acetylcholine. Agents and Actions 6, 642–645.

Juan, H., Sametz, W., 1980. Histamine-induced release of arachidonic acid and prostaglandins in the peripheral vascular bed. Naunyn-Schmiedeberg's Arch. Pharmacol. 314, 183–190.

Juan, H., Sametz, W., 1986. Vasoconstriction induced by noradrenaline and angiotensin II is antagonized by eicosapentaenoic acid independent of formation of trienoic eicosanoids. Naunyn-Schmiedeberg's Arch. Pharmacol. 332, 288–292.

Juan, H., Sametz, W., Saria, A., Pöch, G., 1988. Eicosapentaenoic acid inhibits vasoconstrictor- and noradrenaline-potentiating effects of neuropeptide Y in the isolated rabbit ear. J. Auton. Nerv. Syst. 22, 237–242.

Kernoff, P.B., Willis, A.L., Stone, K.J., Davies, J.A., McNicol, G.P., 1977. Antithrombotic potential of dihomo-gamma-linolenic acid in man. Br. Med. J. 2, 1441–1444.

Kopin, I.J., 1989. Plasma levels of catecholamines and dopamine-β-hydroxylase. In: Trendelenburg, U., Weiner, R. (Eds.), Handbook of Experimental Pharmacology. Springer-Verlag, Berlin, pp. 212–275.

Kromer, B.M., Tippins, J.R., 1996. Coronary artery constriction by isoprostane 8-epi-prostaglandin $F_{2\alpha}$. Br. J. Pharmacol. 119, 1276–1280.

Mallat, Z., Philip, I., Lebret, M., Chatel, D., Maclouf, J., Tedgui, A., 1998. Elevated levels of 8-*iso*-prostaglandin F_2 alpha in pericardial fluid of patients with heart failure: a potential role for in vivo oxidant stress in ventricular dilatation and progression to heart failure. Circulation 16, 1536–1539.

Mancini, G.B., 1996. Emerging concepts: angiotensin-converting enzyme inhibition in coronary artery disease. Cardiovasc. Drugs Ther. 10 (2), 609–612, Suppl.

Möbert, J., Becker, B.F., Zahler, S., Gerlach, E., 1997. Haemodynamic effects of isoprostanes (8-*iso*-prostaglandin $F_{2\alpha}$ and E_2) in isolated guinea pig hearts. J. Cardiovasc. Pharmacol. 29, 789–794.

Morrow, J.D., Roberts, L.J., 1997. The isoprostanes: unique bioactive products of lipid peroxidation. Prog. Lipid Res. 36, 1–21.

Morrow, J.D., Harris, T.M., Roberts, L.J. II, 1990a. Non-cyclooxygenase oxidative formation of a series of novel prostaglandins: analytical ramifications for measurement of eicosanoids. Anal. Biochem. 184, 1–10.

Morrow, J.D., Hill, K.E., Burk, R.F., Nammour, T.M., Badr, K.F., Roberts, L.J. II, 1990b. A series of prostaglandin F_2-like compounds are produced in vivo in humans by a non-cyclooxygenase free radical-catalyzed mechanism. Proc. Natl. Acad. Sci. U.S.A. 87, 9383–9387.

Morrow, J.D., Minton, T.A., Badr, K.F., Roberts, L.J. II, 1994a. Evidence that the F_2-isoprostane, 8-epi-prostaglandin $F_{2\alpha}$, is formed in vivo. Biochem. Biophys. Acta 1210, 244–248.

Morrow, J.D., Minton, T.A., Mukundan, C.R., Campbell, M.D., Zackert, W.E., Daniel, V.C., Badr, K.F., Blair, I.A., Roberts, L.J. II, 1994b. Free radical-induced generation of isoprostanes in vivo. Evidence for the formation of D-ring and E-ring isoprostanes. J. Biol. Chem. 269, 4317–4326.

Morrow, J.D., Roberts, L.J., Daniel, V.C., Awad, J.A., Mirochnitchenko, O., Swift, L.L., Burk, R.F., 1998a. Comparison of formation of D_2/E_2-isoprostanes and F_2-isoprostanes in vitro and in vivo — effects of oxygen tension and glutathione. Arch. Biochem. Biophys. 353 (1997), 160–171.

Morrow, J.D., Scruggs, J., Chen, Y., Zackert, W.E., Roberts, L.J., 1998b. Evidence that the E_2-isoprostane, 15-E_2 *t*-isoprostane (8-*iso*-prostaglandin E_2) is formed in vivo. J. Lipid Res. 39, 1589–1593.

Nicosia, S., Patrono, C., 1989. Eicosanoid biosynthesis and action: novel opportunities for pharmacological intervention. FASEB J. 3, 1941–1948.

Nourooz-Zadeh, J., Halliwell, B., Anggard, E.E., 1997. Evidence for formation of F-3-isoprostanes during peroxidation of eicosapentaenoic acid. Biochem. Biophys. Res. Commun. 236, 467–472.

Pöch, G., Holzmann, S., 1980. Quantitative estimation of overadditive and underadditive drug effects by means of theoretical, additive dose–response curves. J. Pharmacol. Methods 4, 179–188.

Pratico, D., Iuliano, L., Mauriello, A., Spagnoli, L., Lawson, J.A., Maclouf, J., Violi, F., FitzGerald, G.A., 1997. Localization of distinct F_2-isoprostanes in human atherosclerotic lesions. J. Clin. Invest. 100, 2028–2034.

Riegger, G.A., 1996. Role of renin–angiotensin system as a risk factor for control of morbidity and mortality in coronary artery disease. Cardiovasc. Drugs Ther. 10 (2), 613–615, Suppl.

Roberts, L.J., Montine, T.J., Markesbery, W.R., Tapper, A.R., Hardy, P., Chemtob, S., Dettbarn, W.D., Morrow, J.D., 1998. Formation of isoprostane-like compounds (neuroprostanes) in vivo from docosahexaenoic acid. J. Biol. Chem. 273, 13605–13612.

Ross, R., 1986. The pathogenesis of atherosclerosis — an update. New Engl. J. Med. 314, 488–500.

Sametz, W., Prasthofer, S., Krobuschek, T., Wintersteiger, R., Juan, H., 1997. Vascular effects of isoprostanes. Naunyn-Schmiedeberg's Arch. Pharmacol. 356 (1), R7, Suppl.

Sametz, W., Hennerbichler, S., Wintersteiger, R., Juan, H., 1998. Vascular effects of the arachidonic acid (AA)-metabolites 8-epi-$PGF_{2\alpha}$ and 8-epi-PGE_2, the dihomo-γ-linolenic acid (DGLA)-metabolite 8-epi-PGE_1 and the eicosapentaenoic acid (EPA)-metabolite 8-epi-$PGF_{3\alpha}$. Naunyn-Schmiedeberg's Arch. Pharmacol. 358 (2), R718, Suppl.

Schömig, A., Haess, M., Richardt, G., 1991. Catecholamine release and arrhythmias in acute myocardial ischaemia. Eur. Heart J. 12, 38–47.

Schwandt, P., 1991. Effects of Omega-3 Fatty Acids. Schattauer, Stuttgart.

Shaw, A.M., McGrath, J.C., 1996. Initiation of smooth muscle response. In: Garland, C.J., Angus, J.A. (Eds.), Pharmacology of Vascular Smooth Muscle. Oxford Univ. Press, Oxford, pp. 103–135.

Sinzinger, H., Rogatti, W., 1986. Prostaglandin E_1 in Atherosclerosis. Springer-Verlag, Berlin.

Stanton, B.J., Coupar, I.M., 1988. Influence of the thromboxane mimetic U 46619 and the prostacyclin metabolite 6-keto-prostaglandin E_1 on vasoconstriction induced by noradrenaline and 5-HT in rat mesenteric vasculature. Prostaglandin Leukotriene Essent. Fatty Acids 33, 7–12.

Szczeklik, A., Gryglewski, R.J., Sladek, K., Kostka-Trabka, E., Zmuda, A., 1984. Dihomo-gamma-linolenic acid in patients with atherosclerosis: effects on platelet aggregation, plasma lipids and low density lipoprotein-induced inhibition of prostacyclin generation. Thromb. Haemostasis 51, 186–188.

Takahashi, K., Nammour, T.M., Fukunaga, M., Ebert, J., Morrow, J.D., Roberts, L.J. II, Hoover, R.L., Badr, K.F., 1992. Glomerular actions of free-radical-generated novel prostaglandin, 8-epi-prostaglandin $F_{2\alpha}$ in the rat. J. Clin. Invest. 90, 136–141.