The interaction between lipid peroxidation and prostaglandin synthesis in rabbit kidney-medulla slices

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Lipid peroxidation induced by ascorbic acid and Fe^{2+} was inhibited by mepacrine (phospholipase A_2 inhibitor) and aspirin (prostaglandin cyclo-oxygenase inhibitor) in rabbit kidney-medulla slices. Moreover, ascorbic acid and Fe^{2+} potentiated the inhibitory effect on prostaglandin E_2 formation by mepacrine, but they had no influence on prostaglandin E_2 production decreased by aspirin. Lipid peroxidation induced by ascorbic acid and Fe^{2+} appears to be affecting the activity of prostaglandin endoperoxide synthase. These results suggest that lipid peroxidation is connected closely with the prostaglandin-generating system, and it has the potential to modulate the turnover of arachidonic acid and prostaglandin synthesis.

The prostaglandin precursor arachidonic acid is found in cells and tissues esterified mainly to phospholipids. Thus prostaglandin generation under physiological conditions must be preceded by a lipolytic process to release free arachidonic acid from tissue lipids. In a biological system, polyunsaturated fatty acids in phospholipids of membranes are utilized as a substrate for lipid peroxidation. Robak & Sobańska (1976) found that in tissues rich in prostaglandin synthase malondialdehyde is formed mainly from cyclic endoperoxides.

We have reported that lipid peroxidation induced by ascorbic acid and Fe^{2+} enhanced the release of arachidonic acid, but inhibited medullary generation of prostaglandin E (Fujimoto & Fujita, 1982). These studies suggested that an interrelationship might exist between lipid peroxidation and prostaglandin synthesis in renal medulla.

In this present paper we have further examined the interaction between lipid peroxidation and prostaglandin synthesis in rabbit kidney-medulla slices.

Materials and methods

Tissue

Male rabbits (2–2.5 kg body wt.) were used in the present study. The kidneys were removed from anaesthetized (sodium pentobarbital, 30 mg/kg) rabbits and rapidly chilled in ice-cold 0.9% NaCl. The kidney medulla was cut into slices (about 1 mm thick) with a razor blade on an ice-cold Petri dish.

Incubation of medulla slices

In all experiments, rabbit kidney-medulla slices (0.4 g) were preincubated in 4.0 ml of 0.15 M-KCl/20 mM-Tris/HCl buffer, pH 7.4, at 4°C for 5 min. After preincubation, the medium was discarded, and the slices were rinsed twice with the Tris/HCl buffer and incubated for 30 min at 37°C. The medium was then analysed for prostaglandin E_2 and free fatty acids.

Determination of lipid peroxides

Ascorbic acid (1 mM) and $FeSO_4$ (0.4 mM) were added to the medium. Lipid peroxides in kidney-medulla slices were assayed by the thiobarbituric acid reaction, as described previously (Fujimoto & Fujita, 1982). Malondialdehyde was expressed as the thiobarbituric acid values (A_{532}/g of tissue).

Measurement of prostaglandin formation

After incubation, the medium was assayed for prostaglandin E_2 content by a high-pressure liquid-chromatography method (Fujimoto & Fujita, 1982). Briefly, prostaglandin E_2 extracted with ethyl acetate (approx. pH 3) was measured after its base-catalysed conversion into prostaglandin B_2 (Jouvenaz *et al.*, 1970). Peak heights were measured for the quantification of the extracted prostaglandin B_2 relative to a prostaglandin B_2 standard prepared from authentic prostaglandin E_2. This method cannot be used to distinguish between prostaglandins E_1 and E_2. In preliminary studies a portion of the medium was extracted with ethyl acetate and

separated by t.l.c. The ethyl acetate phase was
evaporated to dryness at a room temperature below
30°C under a stream of N_2. The dried material was
dissolved in 0.5 ml of ethanol and then subjected to
t.l.c. on Silica Gel G impregnated with 3% (w/v)
$AgNO_3$. The A II solvent system described by Green
& Samuelsson (1964) was used as a developing
solvent. Authentic prostaglandins E_1, E_2 and $F_{2\alpha}$
were treated similarly, chromatographed concur-
rently and made visible by spraying the plate with
10% (w/v) molybdic acid followed by heating. The
t.l.c. chromatogram indicated that the major prosta-
glandin produced in our incubation and recovered in
the medium is prostaglandin E_2. This observation is
in accord with the data of Erman & Raz (1979),
who reported that the major prostaglandin product
(64–76%) generated by kidney-medulla slices was
prostaglandin E_2, and that the other prostaglandin
products were prostaglandin $F_{2\alpha}$ (17–24%) and
prostaglandin D_2 (6–12%). Thus, in our experi-
ment, measurement of prostaglandin B by the high-
pressure liquid-chromatography method provides an
accurate measurement of the concentration of
prostaglandin E_2 in the medium.

Fatty acid analysis

The individual non-esterified fatty acids in the
incubation medium were measured by a previously
reported method (Yasuda et al., 1980). To separ-
ate the lipid fractions (free fatty acids, triacyl-
glycerols etc.), the total lipids extracted with chloro-
form/methanol (2:1, v/v) were applied on a t.l.c.
plate (Silica Gel H, 0.5 mm thick; Merck) under a
stream of N_2 and then developed with light
petroleum (b.p. 30–37°C)/diethyl ether/acetic acid
(80:30:1, by vol.). The zone containing non-esteri-
fied fatty acids was extracted, and methyl esters
were prepared and quantitatively determined by
g.l.c.

Statistics

The values are presented as means ± S.E.M.
Statistical significance was determined by Student's t
test.

Results

Fig. 1 shows the effects of mepacrine and aspirin
on prostaglandin E_2 synthesis in rabbit kidney-
medulla slices. The antimalarial synthetic drug
mepacrine (quinacrine) has been reported to inhibit
phospholipase A_2 in guinea-pig spleen (Flower &
Blackwell, 1976), rabbit platelets (Vargaftig, 1977),
toad bladder (Yorio & Bentley, 1978) and rat
reticulocytes (Hirata et al., 1979). Aspirin was
previously shown to interfere directly with the
formation of the endoperoxides prostaglandin G_2
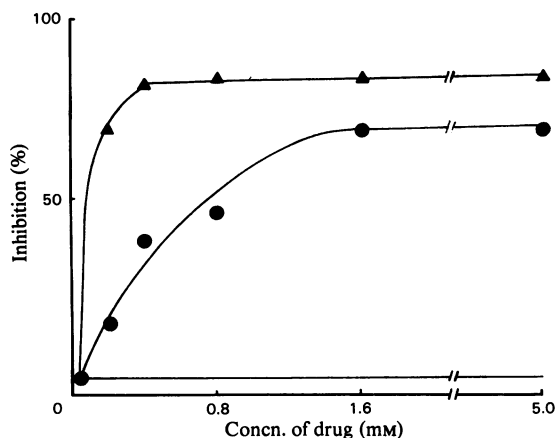and prostaglandin H_2 from arachidonic acid by

Fig. 1. *Effects of mepacrine and aspirin on prosta-
glandin E_2 synthesis in rabbit kidney-medulla slices*
Slices were incubated for 30 min at 37°C in
0.15 M-KCl/20 mM-Tris/HCl buffer in the presence
of different concentrations of mepacrine (●) or
aspirin (▲). Each point represents the mean for three
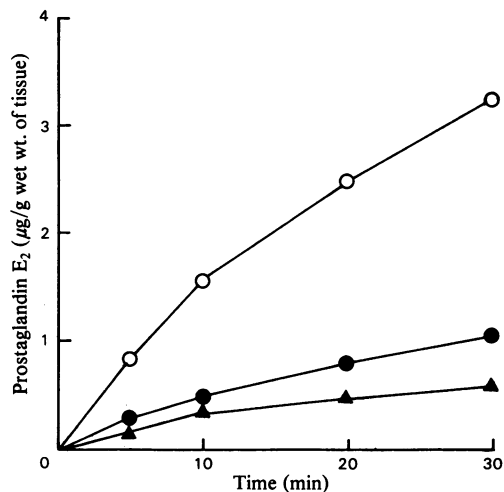experiments.

Fig. 2. *Time course of mepacrine-induced and aspirin-
induced decreases in prostaglandin E_2 release from rabbit
kidney-medulla slices*
Slices were incubated for 30 min at 37°C in
0.15 M-KCl/20 mM-Tris/HCl buffer. Each point rep-
resents the mean for three experiments. ○, Control;
●, 1.6 mM-mepacrine; ▲, 0.4 mM-aspirin.

interacting with prostaglandin cyclo-oxygenase
(Vane, 1971; Miyamoto et al., 1976). To test for
inhibition of prostaglandin synthesis, various con-
centrations of mepacrine or aspirin were added to
the incubation medium. Mepacrine treatment de-
creased basal prostaglandin E_2 production by 70%

at a concentration of 1.6 mM, and aspirin treatment caused inhibition of prostaglandin E_2 production by 82% at 0.4 mM. Increasing the concentrations of mepacrine and aspirin up to 5 mM did not produce further decreases in prostaglandin E_2 production; the concentrations of 1.6 mM-mepacrine and 0.4 mM-aspirin were selected as optimal for further experiments.

The time courses of mepacrine-induced and aspirin-induced decreases in prostaglandin E_2 release from rabbit kidney-medulla slices are shown in Fig. 2. The effects of mepacrine (1.6 mM) and aspirin (0.4 mM) were apparent within 5 min after addition to the incubation mixtures, and they persisted for 30 min. In addition, we confirmed by the use of t.l.c. that the prostaglandin produced under conditions of mepacrine and aspirin inhibition was mainly prostaglandin E_2. These results suggest that the differences between the inhibitory effects of mepacrine and aspirin may be due to differing modes of action rather than to differing speeds at which they work.

To clarify the interaction between lipid peroxidation and prostaglandin synthesis in rabbit kidney medulla, we examined the effects of additions of mepacrine and aspirin during the incubation of kidney-medulla slices with ascorbic acid (1 mM) and Fe^{2+} (0.4 mM). The addition of mepacrine (1.6 mM) or aspirin (0.4 mM) resulted in an inhibition of the lipid peroxidation induced by ascorbic acid and Fe^{2+}, by 61% and 30% respectively (Table 1). On the other hand, ascorbic acid and Fe^{2+} potentiated the inhibitory effect by mepacrine on prostaglandin E_2 formation, but they had no influence on prostaglandin E_2 production decreased by aspirin.

Furthermore, we determined the effects of aspirin and mepacrine on the release of fatty acids from kidney-medulla slices (results not shown). Aspirin (0.4 mM) increased the release of only arachidonic acid approx. 1.6-fold compared with the control, and did not affect the release of other fatty acids (including linoleic acid). This compound therefore appears to exert its effect only on the prostaglandin

cyclo-oxygenase (Vane, 1971). In contrast, mepacrine (1.6 mM) decreased the release of arachidonic acid by about 25.5%.

Previous studies have shown that renal-medullary lipid droplets (Bojesen, 1974; Comai et al., 1975) and medullary tissue (Danon et al., 1975) contain large amounts of triacylglycerols. As shown in Fig. 1, kidney-medullary generation of prostaglandin E_2 was not completely inhibited by mepacrine even at the maximal concentration. In addition, when the total lipids of the incubation medium were chromatographed on a silica-gel t.l.c. plate, large amounts of triacylglycerols were observed. Therefore, to examine the possibility that triacylglycerols could supply the substrate for prostaglandin synthesis, the fatty acid composition of triacylglycerols extracted from various rabbit organs was studied.

Various rabbit organs (kidney cortex, kidney medulla, liver and adipose tissue) were homogenized in 0.15 M-KCl/20 mM-Tris/HCl buffer, and the fatty acid compositions of the triacylglycerol fractions isolated by t.l.c. were determined by g.l.c. The analyses in Table 2 show that the triacylglycerols of kidney cortex and medulla are rich in arachidonic acid as compared with liver and adipose tissue. This result suggests that triacylglycerols rich in arachidonic acid, as well as phospholipids, are able to supply the substrate for the prostaglandin-generating system of rabbit kidney.

Table 2. *Fatty acid compositions of triacylglycerols extracted from various rabbit organs*
The results of duplicate analyses of various pooled organs from five animals are shown.

	Fatty acid composition (%)				
	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{20:4}$
Kidney cortex	41.1	10.1	12.1	2.7	34.1
Kidney medulla	49.2	17.9	0.8	5.1	26.9
Liver	51.9	6.6	24.5	15.0	2.0
Adipose tissue	30.3	9.3	27.1	32.0	1.4

Table 1. *Effects of additions of mepacrine and aspirin during the incubation of kidney-medulla slices with ascorbic acid + Fe^{2+}*
Slices were incubated for 30 min at 37°C in 0.15 M-KCl/20 mM-Tris/HCl buffer. Values are the means ± S.E.M. for five experiments. *$P < 0.01$ compared with corresponding value with ascorbic acid + Fe^{2+}.

Treatment	Thiobarbituric acid value (A_{532}/g of tissue)	Prostaglandin E_2 released (μg/g wet wt. of tissue)
Control	0.27 ± 0.01	3.24 ± 0.26
Ascorbic acid (1 mM) + Fe^{2+} (0.4 mM)	1.70 ± 0.08	1.73 ± 0.15
Mepacrine (1.6 mM)	0.22 ± 0.01	0.88 ± 0.09
Ascorbic acid + Fe^{2+} + mepacrine (1.6 mM)	0.66 ± 0.01*	0.65 ± 0.08
Aspirin (0.4 mM)	0.20 ± 0.02	0.67 ± 0.09
Ascorbic acid + Fe^{2+} + aspirin (0.4 mM)	1.19 ± 0.16*	0.66 ± 0.12

Discussion

The purpose of the present paper is to describe the interaction between lipid peroxidation and the prostaglandin-generating system in rabbit kidney medulla. Robak & Sobańska (1976) found that, in tissues rich in prostaglandin synthase, malondialdehyde is formed mainly from cyclic endoperoxides. Therefore the effects of inhibitors of prostaglandin synthesis on lipid peroxidation induced by ascorbic acid and Fe^{2+} were studied with rabbit kidney-medulla slices.

As shown in Table 1, mepacrine (1.6 mM) and aspirin (0.4 mM) inhibited the lipid peroxidation induced by ascorbic acid and Fe^{2+} by 61% and 30% respectively. The inhibition of malondialdehyde formation by mepacrine in our experiments might be due to the following factors. (1) Mepacrine might protect unsaturated fatty acids from initiation and propagation of lipid peroxidation by binding to phospholipids in the membranes (Nagai et al., 1981), thereby preventing the formation of malondialdehyde from linear hydroperoxides of fatty acids. (2) Mepacrine might decrease the release of the prostaglandin precursor, arachidonic acid, resulting in depression of malondialdehyde formation from cyclic endoperoxides. On the other hand, it is conceivable that aspirin (0.4 mM) decreases malondialdehyde production from cyclic endoperoxides by inhibiting prostaglandin synthesis more powerfully than do 1 mM-ascorbic acid and 0.4 mM-Fe^{2+} (Table 1).

These results suggest that lipid peroxidation is connected closely with the prostaglandin-generating system in kidney medulla, especially at the substrate (arachidonic acid) level and the cyclic endoperoxide level in the pathway of prostaglandin synthesis.

The endogenous biosynthesis of prostaglandins is a measure of the combined activities of the lipase(s), which release arachidonic acid from esterified lipids, and the prostaglandin synthase enzymes, which convert this acid into prostaglandin products. We have previously shown that, in rabbit kidney-medulla slices, lipid peroxidation induced by ascorbic acid and Fe^{2+} enhances the release of arachidonic acid, but inhibits the synthesis of prostaglandin. The lipid peroxidation induced by ascorbic acid and Fe^{2+} thus appears to inhibit prostaglandin synthase.

The present study showed that ascorbic acid and Fe^{2+} potentiated the inhibitory effect on prostaglandin E_2 formation by mepacrine (phospholipase A_2 inhibitor), but that they had no influence on prostaglandin E_2 production decreased by aspirin (prostaglandin cyclo-oxygenase inhibitor) (Table 1). The prostaglandin E_2 is generated from prostaglandin H_2 by a separate isomerase enzyme (Miyamoto et al., 1974). However, the inability of peroxidation to inhibit aspirin-insensitive prostaglandin E_2 generation suggests that this isomerase-catalysed reaction could not have contributed to the inhibitory effect of peroxidation on prostaglandin synthesis. From these observations, the lipid peroxidation induced by ascorbic acid and Fe^{2+} appears to be mainly inhibiting prostaglandin endoperoxide synthase, thereby decreasing prostaglandin E_2 formation.

This speculation is, at least partially, supported by the observation by Egan et al. (1976), who showed that prostaglandin cyclo-oxygenase was irreversibly deactivated by radicals, possibly hydroxyl radicals. The polyunsaturated fatty acids from free-radical intermediates during lipid peroxidation. These free radicals react with protein, and with thiol and non-thiol enzymes (Lewis & Wills, 1962; Chio & Tappel, 1969; Tappel, 1973). Thus free radicals produced by ascorbic acid and Fe^{2+} during lipid peroxidation may de-activate prostaglandin cyclo-oxygenase. Further investigations on endoperoxide production (i.e. the formation of prostaglandins G_2 and H_2) and a marginal prostaglandin E_2 production that cannot be detected by the method used could confirm this hypothesis.

Arachidonic acid released from phospholipids and triacylglycerols is a normal substrate for prostaglandin formation, esterification to other lipids, elongation, desaturation, (in mitochondria) oxidation and lipid peroxidation. The mechanisms of regulation remain to be investigated; however, the present study serves to emphasize that lipid peroxidation has the potential to modulate the turnover of arachidonic acid and prostaglandin synthesis.

References

Bojesen, I. (1974) *Lipids* **9**, 835–843

Chio, K. S. & Tappel, A. L. (1969) *Biochemistry* **8**, 2827–2832

Comai, K., Farber, S. J. & Paulsrud, J. R. (1975) *Lipids* **10**, 551–561

Danon, A., Heimberg, M. & Oates, J. A. (1975) *Biochim. Biophys. Acta* **388**, 318–330

Egan, R. W., Paxton, J. & Kuehl, F. A., Jr. (1976) *J. Biol. Chem.* **251**, 7329–7335

Erman, A. & Raz, A. (1979) *Biochem. J.* **182**, 821–825

Flower, R. J. & Blackwell, G. J. (1976) *Biochem. Pharmacol.* **25**, 285–291

Fujimoto, Y. & Fujita, T. (1982) *Biochim. Biophys. Acta* **710**, 82–86

Green, G. & Samuelsson, B. (1964) *J. Lipid Res.* **5**, 117–120

Hirata, F., Strittmatter, W. J. & Axelrod, J. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 368–372

Jouvenaz, G. H., Nugteren, D. H., Beerthuis, R. K. & Van Dorp, D. A. (1970) *Biochim. Biophys. Acta* **202**, 231–234

Lewis, S. E. & Wills, E. D. (1962) *Biochem. Pharmacol.* **11**, 901–912

Miyamoto, T., Yamamoto, S. & Hayaishi, O. (1974) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3645–3648

Miyamoto, T., Ogino, N., Yamamoto, S. & Hayaishi, O. (1976) *J. Biol. Chem.* **251**, 2629–2636

Nagai, J., Tanaka, M., Hibasami, H. & Ikeda, T. (1981) *J. Biochem. (Tokyo)* **89**, 1143–1148

Robak, J. & Sobańska, B. (1976) *Biochem. Pharmacol.* **25**, 2233–2236

Tappel, A. L. (1973) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **32**, 1870–1874

Vane, J. K. (1971) *Nature (London) New Biol.* **231**, 232–235

Vargaftig, B. B. (1977) *J. Pharm. Pharmacol.* **29**, 222–228

Yasuda, M., Fujita, T., Higashio, T., Okahara, T., Abe, Y. & Yamamoto, K. (1980) *Pflügers Arch.* **385**, 111–116

Yorio, T. & Bentley, P. J. (1978) *Nature (London)* **271**, 79–81