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Inhibition of prostaglandin synthesis in rabbit kidney medulla slices by antioxidants

YOHKO FUJIMOTO*, HIDETOSHI TANIOKA, EIKO TOIBANA, TAKU YAMAMOTO, TADASHI FUJITA, *Department of Hygienic Chemistry, Osaka College of Pharmacy, Matsubara, Osaka 580, Japan*

The inhibitory effects of *NN'*-diphenyl-*p*-phenylenediamine (DPPD), sodium diethyldithiocarbamate (SDDC) and 2,6-di-t-butylphenol (DTBP) on the generation of medullary prostaglandin E have been compared. DPPD (1 μM) and SDDC (1 mM) failed to inhibit arachidonic acid-induced stimulation of prostaglandin E synthesis, while DTBP (1 mM) inhibited it. This suggests that DPPD and SDDC inhibit prostaglandin formation by affecting a phospholipase pathway, and the inhibition of prostaglandin generation by DTBP occurs at the cyclooxygenase step.

We have shown that phospholipase A_2 activity in rat liver mitochondria is stimulated by lipid peroxidation by ascorbic acid and Fe^{2+} (Yasuda & Fujita 1977), and lipid peroxidation also enhances the release of arachidonic acid from rabbit kidney medulla slices, although it inhibits the synthesis of prostaglandin E (PGE) in this tissue (Fujimoto & Fujita 1982; Fujimoto et al 1983). It can be conceived that lipid peroxidation has the potential to modulate the turnover of arachidonic acid and prostaglandin synthesis. Recently, we reported that antioxidants such as *NN'*-diphenyl-*p*-phenylenediamine (DPPD), sodium diethyldithiocarbamate (SDDC) and 2,6-di-t-butylphenol (DTBP) inhibit the generation of medullary PGE (Fujita et al 1982). This study was undertaken to investigate the mechanisms by which these antioxidants inhibit PGE synthesis in kidney medulla.

Materials and methods

Kidney medulla slices were prepared from male rabbits (2–2·5 kg) as described by Fujimoto & Fujita (1982). Medulla slices (0·4 g) were preincubated in 4·0 ml 0·15 M KCl/0·02 M Tris-HCl buffer (pH 7·4) for 5 min at 4 °C. Following preincubation, the medium was discarded, the slices rinsed twice with the Tris-HCl buffer and incubated for 30 min at 37 °C. In individual experiments, we added to the incubation medium: mepacrine (1·6 mM), aspirin (0·4 mM), DPPD (1 μM), SDDC or DTBP (1 mM). Lower concentrations of the antioxidants were not effective on medullary prostaglandin synthesis (Fujita et al 1982).

After incubation, the medium was assayed for PGE content by hplc described by Fujimoto et al (1983). Briefly, PGE extracted with ethyl acetate (approximately pH 3) was measured after its base-catalysed conversion to prostaglandin B (Jouvenaz et al 1970). Peak heights were measured for the quantification of the extracted prostaglandin B relative to a prostaglandin B_2 standard prepared from authentic prostaglandin E_2.

* Correspondence.

Results and discussion

The time course of DPPD-, SDDC- and DTBP-induced decreases in PGE release from rabbit kidney medulla slices is shown in Fig. 1. The effects of DPPD (1 μM), SDDC and DTBP (1 mM) were apparent within 5 min after addition to the incubation mixture, and they persisted for 30 min.

Some peroxides are required for the activation of prostaglandin-forming cyclooxygenase (Lands et al 1971; Smith & Lands 1972). In addition, lipid peroxidation is connected closely with phospholipase A_2 (Yasuda & Fujita 1977; Fujimoto & Fujita 1982). Thus, inhibition of PGE synthesis by antioxidants seems to occur at either the cyclooxygenase or phospholipase reaction. To distinguish between these two possible loci, prostaglandin synthesis was stimulated by incubating the slices in the presence of arachidonic acid (Fig. 2).

After slices were incubated for 30 min at 37 °C with

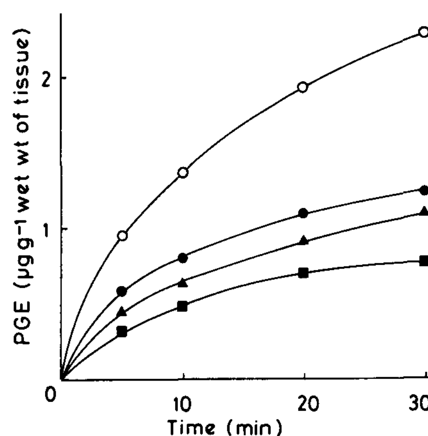

FIG. 1. Time course of *NN'*-diphenyl-*p*-phenylenediamine (DPPD)-, sodium diethyldithiocarbamate (SDDC)- and 2,6-di-t-butylphenol (DTBP)-induced decreases in PGE release from rabbit kidney medulla slices. Slices were incubated for 30 min at 37 °C in 0·15 M KCl/0·02 M Tris-HCl buffer. ○, control; ●, 1 μM DPPD; ▲, 1 mM DTBP; ■, 1 mM SDDC. Each point represents the mean of 3 experiments.

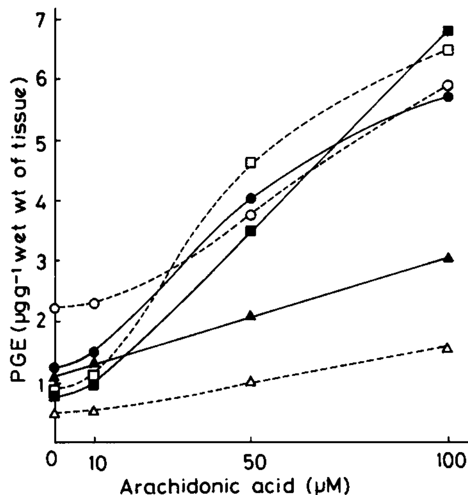

FIG. 2. Effects of *NN'*-diphenyl-*p*-phenylenediamine (DPPD), sodium diethyldithiocarbamate (SDDC) and 2,6-di-t-butylphenol (DTBP) on stimulation of PGE production by exogenous arachidonic acid. Slices were incubated for 30 min at 37 °C in 0·15 M KCl/0·02 M Tris-HCl buffer with varying amounts of arachidonic acid. ○, no reagent added; △, 0·4 mM aspirin; □, 1·6 mM mepacrine; ●, 1 µM DPPD; ▲, 1 mM DTBP; ■, 1 mM SDDC. Each point represents the mean of 5 experiments.

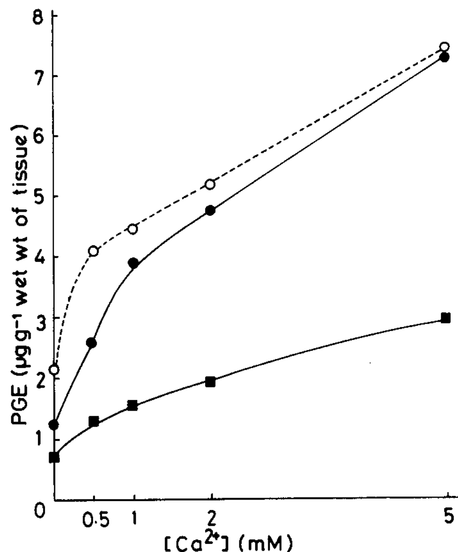

FIG. 3. Effects of *NN'*-diphenyl-*p*-phenylenediamine (DPPD) and sodium diethyldithiocarbamate (SDDC) on stimulation of PGE production by exogenous Ca^{2+}. Slices were incubated for 30 min at 37 °C in 0·15 M KCl/0·02 M Tris-HCl buffer with varying amounts of Ca^{2+}. ○, no reagent added; ●, 1 µM DPPD; ■, 1 mM SDDC. Each point represents the mean of 5 experiments.

varying amounts of arachidonic acid there was a dose-dependent stimulation of PGE production. Mepacrine, which inhibits phospholipase A_2 (Flower & Blackwell 1976) at 1·6 mM, and aspirin, which interferes directly with the formation of the endoperoxides PGG_2 and PGH_2 from arachidonic acid by interacting with prostaglandin cyclooxygenase (Vane 1971; Miyamoto et al 1976), 0·4 mM, reduced the production of basal PGE, 63 and 80% respectively.

In the presence of arachidonic acid (50, 100 µM) the arachidonic acid-induced stimulation of PGE formation was not blocked by mepacrine, DPPD or SDDC. However, at three arachidonic acid concentrations the inhibition caused by aspirin or DTBP was constant at 78 ± 2% or 55 ± 2%. These results indicate that DPPD and SDDC inhibit the deacylation of phospholipids (and the availability of arachidonic acid) by perhaps inhibiting a phospholipase pathway rather than by affecting the cyclooxygenase enzyme, and that the inhibition of prostaglandin generation by DTBP is occurring primarily at the cyclooxygenase step in the metabolism of arachidonic acid.

It seems likely that DTBP, which could act to remove peroxides, may have an inhibitory effect by keeping the cyclooxygenase in an inactive form. On the other hand, Dise et al (1982) reported that mepacrine interacted directly with membrane phospholipids, primarily phosphatidylethanolamine, to form less polar derivatives. Mepacrine-phospholipid interaction could alter membrane architecture and disrupt membrane protein-lipid interactions. We have previously shown that phospholipase A_2 seemingly bound to the membrane structures is released by alteration in the phospholipid structure of the membranes after lipid peroxidation (Yasuda & Fujita 1977). It is considered that DPPD and SDDC could protect the membrane phospholipid and reduce peroxide levels, resulting in the disruption of phospholipase-lipid interaction.

Phospholipase A_2 characteristically requires Ca^{2+} as an obligatory cofactor (Brockerhoff & Jensen 1974; Van den Bosch 1980). Fig. 3 compares the effects of DPPD and SDDC on stimulation of PGE production by exogenous Ca^{2+}. Ca^{2+} stimulated PGE production in a dose-dependent manner. With DPPD, the degree of inhibition was seen to vary with the concentrations of Ca^{2+}. In the presence of 5 mM Ca^{2+}, DPPD failed to inhibit PGE production induced by Ca^{2+}. In contrast, the inhibition caused by SDDC was nearly constant in the presence of varying amounts of Ca^{2+}. These results are difficult to explain since little is known of the effect of DPPD and SDDC on enzymes in membranes. However, the most feasible explanations are that Ca^{2+} also is able to alter the properties of membrane surface (Ohki et al 1981), and that SDDC and interestingly its dimer disulfiram might have a strong chelating action as well as an antioxidative action.

This report shows that the inhibition of PGE synthesis by antioxidants appears to be either via affecting the cyclooxygenase enzyme (DTBP) or by inhibiting a

phospholipase pathway (DPPD, SDDC). Thus, these three antioxidants could act to reduce peroxide levels and inhibit PGE synthesis by different mechanisms. It is possible that the differences may be related to differing modes of antioxidative action depending on the type of antioxidants.

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Amitriptyline and femoxetine, but not clomipramine or citalopram, antagonize hyperthermia induced by directly acting 5-hydroxytryptamine-like drugs in heat adapted rats

LESZEK PAWŁOWSKI, *Institute of Pharmacology, Polish Academy of Sciences, 12 Smętna Street, 31-343 Kraków, Poland*

5-HT uptake inhibitors and pirenperone (a 5-HT_2 receptor antagonist), which in previous experiments antagonized fenfluramine (5-HT releaser)-induced hyperthermia in heat adapted rats, were tested against hyperthermia induced by the directly acting 5-HT agonist—*m*-CPP and quipazine. Pirenperone and—to a lesser degree—amitriptyline and femoxetine antagonized the hyperthermia. Citalopram and clomipramine were inactive. It is concluded that hyperthermia induced by 5-HT-like drugs in rats is due to the stimulation of the 5-HT_2 receptor and that the antagonistic effect of citalopram and clomipramine against fenfluramine-induced hyperthermia might be connected with their effect on the uptake of 5-HT.

5-Hydroxytryptamine (5-HT) uptake inhibitors, amitriptyline, clomipramine, femoxetine and citalopram, prevent hyperthermia induced by the 5-HT releasing agent, fenfluramine, in rats housed at 26–28 °C (Frey 1975; Sulpizio et al 1978; Pawłowski 1981; Sugrue 1981; Maj et al 1982). This hyperthermia is central in origin and has been proved to be mediated by 5-HT (Sulpizio et al 1978). Therefore, the above fact is in agreement with the view that 5-HT uptake inhibitors can block the entrance of fenfluramine into the 5-HT-ergic neurons (via inactivation of the neuronal membrane carrier system) and in this way prevent the pharmacological action of this drug (Ghezzi et al 1973; Frey 1975; Sulpizio et al 1978). However, there is a poor correlation between degree of the inhibition of the 5-HT uptake and the protection against the fenfluramine-induced hyperthermia for the 5-HT uptake inhibitors (Sugrue 1981; Maj et al 1982). Moreover, some potent 5-HT uptake inhibitors (e.g. zimelidine and Org 6582) do not prevent fenfluramine-induced hyperthermia (Pawłowski et al 1980; Pawłowski 1981; Sugrue 1981). Therefore, it could be suggested that the antagonistic action of amitriptyline, clomipramine, femoxetine and citalopram is due to a mechanism different from 5-HT uptake inhibition, i.e. the blockade of the carrier system for 5-HT and fenfluramine. Indeed, the anti-5-HT action of amitriptyline has been observed in many other pharmacological tests (Fuxe et al 1977; Maj et al 1979; Kwiatek et al 1980; Hall & Ögren 1981), and femoxetine can antagonize the 5-HT receptor in the blood vessels (Petersen et al 1979). So far, the central anti-5-HT action of clomipramine cannot be excluded (Hall & Ögren 1981) and only citalopram has never been suggested to possess central or peripheral anti-5-HT properties (Hyttel 1982). In this context, it seemed interesting to investigate the effects of amitriptyline, clomipramine, femoxetine and citalopram upon the hyperthermia induced by the directly acting 5-HT-mimetics, 1-(m-chlorophenyl)-piperazine (m-CPP) and quipazine (Maj & Lewandowska 1980). Pirenperone dihydrochloride (R 50 656), a new potent 5-HT (5-HT_2) receptor blocking agent (Colpaert & Leysen 1981; Colpaert et al 1982; Krstić & Katušić 1982; Leysen et al 1982), which in the preliminary experiments (Pawłowski, to be published), in low doses