J. Pharm. Pharmacol. 1987, 39: 314–315
Communicated September 17, 1986

Effect of pyridoxine on prostaglandin synthesis in rabbit kidney medulla slices

YOHKO FUJIMOTO*, KAZUYUKI NISHIOKA, NOBORU OHTANI, MIDORI AIHARA, TADASHI FUJITA, *Department of Hygienic Chemistry, Osaka University of Pharmaceutical Sciences, Kawai, Matsubara, Osaka 580, Japan*

Pyridoxine stimulated the generation of prostaglandin E_2 in rabbit kidney medulla slices. Moreover, pyridoxine in the presence of aspirin reduced the release of linoleic acid, but enhanced the release of arachidonic acid from the medulla slices as compared with aspirin alone, indicating that the enhancement of prostaglandin E_2 formation elicited by pyridoxine may be ascribed to an increased conversion of linoleic acid to arachidonic acid. These results suggest that pyridoxine can be an important modulating factor in prostaglandin synthesis by the kidney.

Of all the fatty acids that occur combined with glycerol as triacylglycerols in foods, only a small number are essential for the nutritional well being of animals, including man. These fatty acids are polyunsaturated, and have a specific structure that cannot by synthesized by animals. Linoleic acid (18 : 2 ω6) is probably the most abundant of these essential polyunsaturated fatty acids. It occurs in high concentrations in many vegetable oils, e.g., corn, cottonseed, safflower, and soybean. The prostaglandin precursor, arachidonic acid (20 : 4 ω6), occurs in small amounts in animal lipids; however, this essential fatty acid can be synthesized from linoleic acid. According to Mead (1961), linoleic acid was transformed into arachidonic acid by elongation and desaturation. Witten & Holman (1952), after experimenting with rats, concluded that pyridoxine was involved in the conversion of linoleic acid to arachidonic acid. Nine years later, Wakil (1961) showed that the relationship between pyridoxine and essential fatty acid metabolism might be confined to the role of pyridoxal phosphate in the elongation of fatty acids.

Very little work has been done on the regulation of lipid composition and lipid metabolism in the kidney. The present study was undertaken to investigate the influence of pyridoxine on the in-vitro production of prostaglandin E_2 in kidney medulla and the release of fatty acids from the medulla slices. In this paper we describe our finding that a definite relationship does exist.

Materials and methods

Male rabbits (2–2·5 kg) were anaesthetized (sodium pentobarbitone, 30 mg kg^{-1}) and the kidneys removed then rapidly chilled in ice-cold saline. The kidney medulla slices were prepared as described by Fujimoto & Fujita (1982). In all experiments, slices (0·4 g) were preincubated in 4·0 mL 0·15 M KCl/0·02 M Tris-HCl buffer (pH 7·4) at 4 °C for 5 min. Following preincubation, the medium was discarded, the slices rinsed twice with the Tris-HCl buffer and incubated with various concentrations of pyridoxine at 37 °C for 60 min. After incubation the medium was assayed for prostaglandin E_2 content by an HPLC method (Fujimoto et al 1983). Briefly, prostaglandin E_2 extracted with ethyl acetate (approximately pH 3) was measured after its base-catalysed conversion to 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.

The individual free fatty acids in the incubation medium were measured by a previously reported method (Yasuda et al 1980). To separate the lipid fractions (free fatty acids, triacylglyceros etc.), the total lipids extracted with chloroform–methanol (2 : 1, v/v) were applied on a TLC plate (Silica Gel H, 0·5 mm thick; Merck) under a stream of N_2 and then developed with light petroleum (b.p. 30–70 °C)–diethyl ether–acetic acid (80 : 30 : 1, v/v). The free-fatty-acid zone was extracted, and methyl esters were prepared and quantitatively determined by GLC. The concentration of the individual fatty acids in the medium was evaluated from the peak area calculated by the triangulation, in comparison with the known amounts of margaric acid (15 μg), which appeared as a new peak.

The values presented in this paper are the means ± s.e.m. Statistical significance was calculated using Student's paired *t*-test.

Results and discussion

Fig. 1 illustrates the effects of various concentrations of pyridoxine on prostaglandin E_2 synthesis in rabbit kidney medulla slices. Pyridoxine stimulated medullary generation of prostaglandin E_2 at concentrations ranging from 0·1 to 3 mM. Maximal effect on prostaglandin E_2 production was observed at 0·5 mM pyridoxine. The effect of pyridoxine (0·5 mM) was apparent within 15 min after addition to the incubation mixture and persisted for 60 min (Fig. 2).

Moreover, we determined the effect of pyridoxine in the presence of aspirin on the release of fatty acids from kidney medulla slices (Table 1). Aspirin has been shown to interfere directly with the formation of the endoperoxides prostaglandin G_2 and prostaglandin H_2 from

* Correspondence.

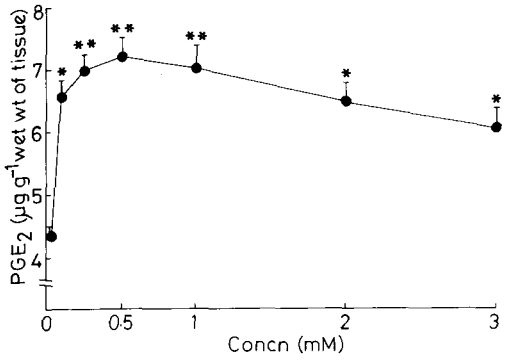

FIG. 1. Effect of pyridoxine on prostaglandin E_2 synthesis in rabbit kidney medulla slices. Slices were incubated for 60 min at 37 °C in 0·15 M KCl/0·02 M Tris-HCl buffer in the presence of different concentrations of pyridoxine. Each point indicates the mean of six experiments; vertical lines show s.e.m. * $P < 0·05$ compared with corresponding value in the absence of pyridoxine. ** $P < 0·01$ compared with corresponding value in the absence of pyridoxine.

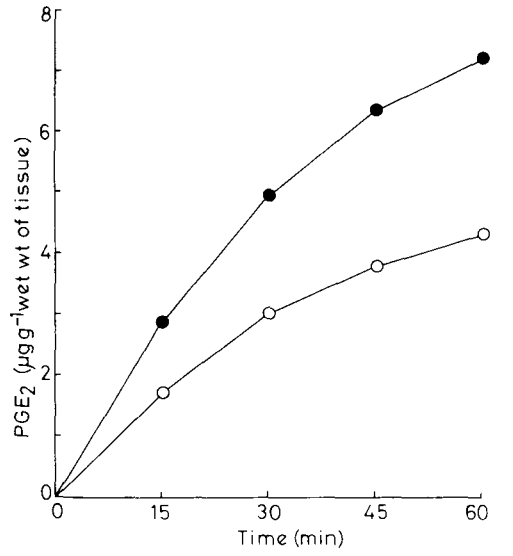

FIG. 2. Time course of prostaglandin E_2 release from rabbit kidney medulla slices. Incubations were for 60 min at 37 °C in 0·15 M KCl/0·02 M Tris-HCl buffer in the absence (○) and the presence of 0·5 mM pyridoxine (●). Each point indicates the mean of five experiments (s.e.m. values were less than 5%).

arachidonic acid by interacting with prostaglandin cyclooxygenase (Vane 1971; Miyamoto et al 1976). We have previously reported that aspirin inhibits basal prostaglandin E_2 production in rabbit kidney medulla slices by 82% and increases the release of only arachidonic acid from the medulla slices at a concentration of 0·4 mM (Fujimoto et al 1983). In the present experiments, aspirin (0·4 mM) alone increased the release of arachidonic acid approximately 1·6-fold compared with the control and did not affect the release of other fatty acids (results not shown). As shown in Table 1, pyridoxine (0·5 mM) in the presence of aspirin (0·4 mM) significantly reduced the release of oleic acid and linoleic acid, but enhanced the release of arachidonic acid 1·4-fold compared with aspirin alone. These results imply that the increased conversion of linoleic acid to arachidonic acid and the resulting increased availability of free arachidonic acid is the main cause for the pyridoxine-induced prostaglandin E_2 formation. Thus, our present data strongly suggest that pyridoxine can be an important modulating factor in prostaglandin biosynthesis by the kidney.

Table 1. Effect of pyridoxine in the presence of aspirin on the release of fatty acids 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 in the presence of 0·4 mM aspirin.

	Fatty acids released (μg g^{-1} wet wt of tissue)				
	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{20:4}$
Aspirin (0·4 mM)	6·27 ± 0·64	3·75 ± 0·38	2·00 ± 0·18	3·44 ± 0·29	0·69 ± 0·07
Aspirin (0·4 mM) + Pyridoxine (0·5 mM)	4·72 ± 0·17	3·33 ± 0·07	1·02 ± 0·17*	1·67 ± 0·13**	0·98 ± 0·10

Values are means ± s.e.m. (n = 3). * $P < 0·05$ compared with aspirin-treated values. ** $P < 0·02$ compared to aspirin-treated values. Abbreviations: $C_{16:0}$, palmitic acid; $C_{18:0}$, stearic acid; $C_{18:1}$, oleic acid; $C_{18:2}$, linoleic acid; $C_{20:4}$, arachidonic acid.

REFERENCES

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

Fujimoto, Y., Tanioka, H., Keshi, I., Fujita, T. (1983) Biochem. J. 212: 167–171

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

Mead, J. F. (1961) Fed. Proc. Fed. Am. Soc. Exp. Biol. 20: 952–955

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

Vane, J. R. (1971) Nature (London) New Biol. 231: 232–235

Wakil, S. J. (1961) J. Lipid Res. 2: 1–24

Witten, P. W., Holman, R. T. (1952) Arch. Biochem. Biophys. 41: 266–273

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