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EFFECT OF POLYAMINES ON PROSTAGLANDIN SYNTHESIS IN VARIOUS CELL-FREE SYSTEMS

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SUMMARY: - Synthesis of $PGF_{2\alpha}$ by bovine uterus and guinea pig lung microsomes and that of TXB_2 by human platelet and rat spleen microsomes were stimulated by spermine. PGE_2 synthesis by bovine seminal vesicle and porcine lung microsomes, and 6-keto-$PGF_{1\alpha}$ synthesis by bovine seminal vesicle and uterus microsomes were inhibited by spermine. When phospholipid-free prostaglandin synthetase from bovine seminal vesicle was used instead of microsomes, the inhibition of PGE_2 synthesis by spermine disappeared. The inhibition of PGE_2 synthesis by spermine gradually appeared with an increase of phospholipid added. Among phospholipids tested, phosphatidylcholine was the most effective for the inhibition of PGE_2 synthesis by spermine.

Polyamines have been implicated in numerous growth processes (1). However, the detailed mechanism by which polyamines are concerned in the cell growth is not well understood. Since polyamines can interact with nucleic acids (2-4), the effects of polyamines on DNA synthesis (5), RNA synthesis (6), and protein synthesis (7) have been studied extensively. Furthermore, interaction between polyamines and membranes has also been suggested from the results that polyamines can prevent the lysis of *Micrococcus luteus* with lysozyme (8) and can change the activity of membrane enzymes (9-11).

Since polyamine synthesis was stimulated by PGE_1 and PGE_2 *via* the increase of cAMP in various cells (12,13), we have intended to study the effect of polyamines on prostaglandin synthesis as an example of the interaction between polyamines and membranes. In this communication, we show that polyamines influence each

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prostaglandin synthesis differently and phospholipids are important for the effect of polyamines.

MATERIALS AND METHODS

Materials - Fresh bovine seminal vesicle and uterus and porcine lung were supplied by Teikoku Hormone Mfg. Co. and stored at -20°C until used. PGA_2 and PGE_2 were purchased from Sigma Chemical Co. PGD_2, $PGF_{2\alpha}$, and 6-keto-$PGF_{1\alpha}$ were the generous gifts of Dr. I. Morita. TXB_2 was kindly supplied by Dr. J. E. Pike. Arachidonic acid and imidazole (an inhibitor of thromboxane synthesis) were obtained from Nakarai Chemicals. [1-^{14}C]Arachidonic acid (specific activity, 56 mCi/mmol) was from Radiochemical Centre Amersham. Thin layer chromatography plates of silica gel $60F_{254}$, 0.25 mm in thickness, were purchased from E. Merck. 3-Hydroperoxy-3-methyl-2-phenyl-3H-indole [an inhibitor of PGI_2 synthesis (14)] was kindly supplied by Dr. M. Nakagawa. [^{3}H]Prostaglandin E radioimmunoassay kit was obtained from Clinical Assays, Inc.

Phospholipids were extracted from rat liver microsomes by the method of Folch et al. (15). Concentration of phospholipids was calculated as twenty five times as much as the amounts of phosphorus. The content of phophorus was determined by the method of Morrison (16). Phosphatidylcholine from egg, phosphatidylethanolamine from bovine brain, phosphatidylcholine dipalmitoyl, and phosphatidylcholine dioleoyl were purchased from Serdary Research Laboratories. Phosphatidylcholine and phosphatidylinositol from soybean were from Sigma Chemical Co. Phospholipids were sonicated with a Bronwill Biosonik IV before use.

Preparation of microsomes - Bovine uterus and seminal vesicle, guinea pig lung, porcine lung, and rat spleen were homogenized with 2.5 volumes of 0.05 M phosphate buffer, pH 7.4. The homogenate was centrifuged at 10,000 x g for 15 min, and the supernatant fraction was centrifuged again at 105,000 x g for 60 min. The microsomal pellet thus obtained was suspended with 0.05 M phosphate buffer, pH 7.4, and kept at -70°C until used. Human platelet microsomes were prepared by the method of Tai and Yuan (17).

Partial purification of prostaglandin synthetase - Prostaglandin synthetase was partially purified from bovine seminal vesicle microsomes according to the method of Miyamoto et al. (18). "Solubilized enzyme" (130 mg protein) prepared by their method was applied to a DEAE-cellulose column (1.8 x 15 cm) previously equilibrated with 20 mM potassium phosphate, pH 7.4, containing 0.2% Tween 20 (Buffer A). The protein was eluted from the column successively with Buffer A, buffer A containing 100 mM KCl, and Buffer A containing 200 mM KCl, to obtain Fraction I, II, and III, respectively. These fractions were all free of phospholipid, and PGE_2 synthetic activity was obtained by the combination of Fractions I and III. Each fraction was treated with Bio-Beads SM2 (Bio•Rad Laboratories) to remove Tween 20 according to the method of Holloway (19).

Assay for prostaglandin synthesizing activity - The reaction mixture (0.5 ml), which contained 0.1 M Bicine-NaOH (pH 8.0), 2.5 mM tryptophan, 1 μM hemin, 1 mM glutathione, 0.1 mM EDTA, 0.2 μCi of [1-^{14}C]arachidonic acid, and bovine seminal vesicle microsomes (100 μg protein) or the microsomes from other sources (800 μg protein), was incubated at 37°C for 30 min with shaking. When micro-

somes from human platelet were used, incubation was carried out at 37°C for 5 min. Reaction was terminated by the addition of an appropriate amount of 1 M HCl to bring the pH of the reaction mixture to 3.0. The radioactive products were extracted with 4 ml of ethyl acetate. The resulting organic phase was evaporated to dryness under reduced pressure. Residues were dissolved in 50 µl of ethanol and a 20 µl aliquot was applied to thin layer chromatography plates. The plates were developed in the organic phase of ethyl acetate/isooctane/acetic acid/water (11:5:2:10, vol/vol) described by Hassid et al. (20), and the radioactive products were detected by radiochromato-scanner. Each radioactive product was separately scraped off and extracted with a mixture of methanol and chloroform (1/1, v/v). The radioactivity was counted by a liquid scintillation counter and the amounts of prostaglandin formed were expressed as pmols per 0.5 ml of the reaction mixture.

RESULTS AND DISCUSSION

Effect of polyamines on prostaglandin synthesis by microsomes - Effect of spermine on prostaglandin synthesis was examined using microsomes from various tissues (Fig. 1). Each prostaglandin was identified by comparing its Rf value with that of the standard. In addition, radioimmunoassay and specific inhibitors (imidazole and 3-hydroperoxy-3-methyl-2-phenyl-3H-indole) were used for the identification of PGE_2, TXB_2, and 6-keto-$PGF_{1\alpha}$. Synthesis of $PGF_{2\alpha}$ was stimulated greatly by spermine in the system containing microsomes from guinea pig lung or bovine uterus. Synthesis of TXB_2 by human platelet and rat spleen microsomes was stimulated also by spermine. Synthesis of PGE_2 by bovine seminal vesicle and porcine lung microsomes and of 6-keto-$PGF_{1\alpha}$ by bovine uterus and seminal vesicle microsomes was inhibited by spermine (Fig. 1). Synthesis of PGD_2 was also inhibited by spermine in the system containing bovine seminal vesicle or rat spleen microsomes.

These results are interesting since TXA_2 (an active precursor of TXB_2) is a platelet aggregating compound while PGI_2 (an active precoursor of 6-keto-$PGF_{1\alpha}$) is an antiaggregating compound (21). As PGE_1, PGE_2 (12,13), and probably PGI_2 (22) are triggers of polyamine biosynthesis via the increase of cAMP content, the inhibition of PGE_2 and 6-keto-$PGF_{1\alpha}$ synthesis by spermine may be a kind of

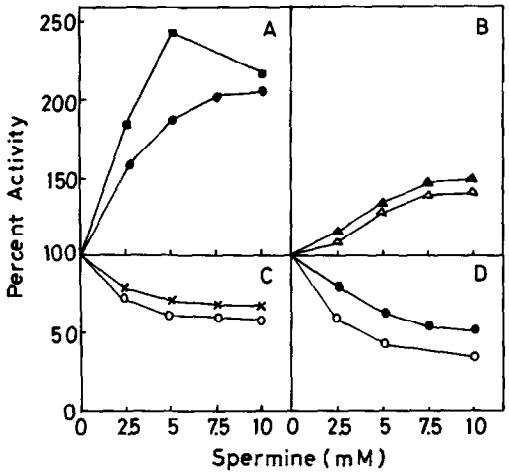

Fig. 1. Effect of spermine on $PGF_{2\alpha}$ (A), TXB_2 (B), PGE2 (C), and 6-keto-$PGF_{1\alpha}$ (D) synthesis by microsomes. Microsomes used were from bovine uterus (●), bovine seminal vesicle (○), guinea pig lung (■), porcine lung (×), human platelet (▲), and rat spleen (△). The amounts of prostaglandin formed without spermine were as follows: $PGF_{2\alpha}$, 43.5 (■) and 32.4 (●) pmol; TXB_2, 232 (▲) and 54.2 (△) pmol; PGE_2, 650 (○) and 84.2 (×) pmol; 6-keto-$PGF_{1\alpha}$, 67.8 (●) and 43.2 (○) pmol.

"feedback inhibition". It may be also rational that polyamines can stimulate the synthesis of $PGF_{2\alpha}$ and TXB_2 which are necessary for cell proliferation (23,24)

Effect of various polyamines was then tested with guinea pig lung and bovine seminal vesicle microsomes (Fig. 2). Spermine was found to be the most effective among polyamines tested and the effective concentration was approximately 5 mM.

Effect of phospholipids and spermine on the synthesis of PGE_2 by phospholipid-free prostaglandin synthetase from bovine seminal vesicle - It was examined whether or not phospholipids are necessary for the inhibition of PGE_2 synthesis by spermine using phospholipid-free prostaglandin synthetase. As shown in Table I, spermine inhibition of PGE_2 synthesis disappeared when phospholipid-free prostaglandin synthetase was used instead of microsomes. When phospholipids were added to the reaction mixture, inhibition of PGE_2 synthesis by spermine gradually appeared with increasing

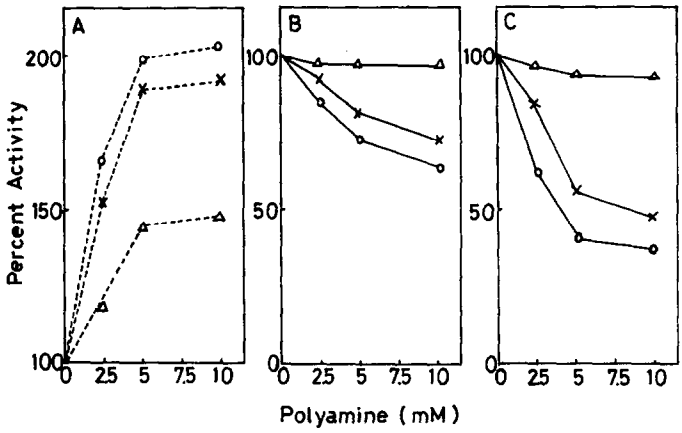

Fig. 2. Effect of polyamines on $PGF_{2\alpha}$ (A), PGE_2 (B), and 6-keto-$PGF_{1\alpha}$ (C) synthesis by guinea pig lung microsomes (-----) or bovine seminal vesicle microsomes (——). o, Spermine; x, spermidine; Δ, putrescine.

amount of phospholipids. Among phospholipids tested, phosphatidylcholine was the most effective for the inhibition of PGE_2 synthesis by spermine (Fig. 3). On the contrary, phosphatidylinositol stimulated PGE_2 synthesis by spermine. Since the content of phosphatidylinositol in membranes is low, the effect

Table I. Effect of phospholipids and spermine on the synthesis of PGE_2 by phospholipid-free prostaglandin synthetase from bovine seminal vesicle.

Phospholipids added (μg)	PGE_2 formed (pmol)		Ratio (5 mM spermine/ without spermine)
	Without spermine	5 mM spermine	
-	515	488	0.95
10	519	454	0.88
20	528	450	0.85
40	460	327	0.73
60	392	206	0.53

For the assay of PGE_2 synthesis, 24 μg of Fraction I and 28 μg of Fraction III were used. Phospholipids from rat liver microsomes were used in this experiment.

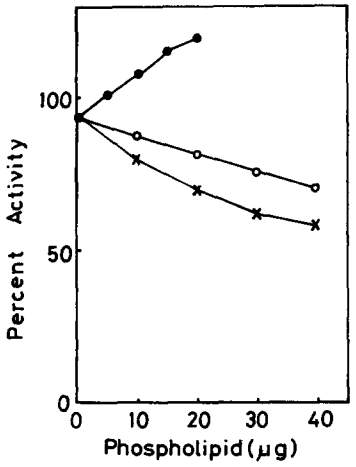

Fig. 3. Effect of phospholipid and spermine on PGE_2 synthesis. For the assay of PGE_2 synthesis, 24 μg of Fraction I and 28 μg of Fraction III were used. •, Phosphatidylinositol; o, phosphatidylethanolamine; x, phosphatidylcholine.

of phosphatidylinositol may be overwhelmed by the effect of phosphatidylcholine in microsomal system. These results suggest that phospholipids are important for the effect of polyamines on prostaglandin synthesis.

Then, influence of fatty acid composition in phosphatidylcholine

Table II. Effect of spermine and phosphatidylcholines on PGE_2 synthesis.

Phosphatidylcholine (20 μg)	Spermine (5 mM)	PGE_2 formed (pmol)	PGE_2 formed (%)	Inhibition by spermine (%)
-	-	520	100	
-	+	494		5
Phosphatidylcholine dipalmitoyl	-	657	126	
	+	434		34
Phosphatidylcholine dioleoyl	-	322	62	
	+	184		43
Phosphatidylcholine from egg	-	312	60	
	+	187		40
Phosphatidylcholine from soybean	-	214	41	
	+	131		39

For the assay of PGE_2 synthesis, 24 μg of Fraction I and 28 μg of Fraction III were used.

was examined. Although percentage inhibition of PGE_2 synthesis by spermine was nearly equal in the presence of different phosphatidylcholines, phosphatidylcholines affected the rate of PGE_2 synthesis differently as the fatty acid composition varied (Table II). It has been reported that the content of unsaturated fatty acids in phosphatidylcholine from soybean was higher than that in phosphatidylcholine from egg (25,26). Therefore, it is suggested that unsaturated fatty acids in phospholipids inhibit PGE_2 synthesis, although phosphatidylcholine containing saturated fatty acid stimulated PGE_2 synthesis (Table II).

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