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EFFECT OF PGI_2 ON TRANSCELLULAR TRANSPORT OF FLUORESCEIN DEXTRAN THROUGH AN ARTERIAL ENDOTHELIAL MONOLAYER

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Abstract—The effects of prostacyclin (PGI_2) and stable derivatives of PGI_2, such as isocarbacyclin (PGI_2 deriv. (A)) and isocarbacyclin methyl ester (PGI_2 deriv. (B)), on junctional transport of fluorescein dextran (FD) through cultured porcine arterial endothelial cells were investigated. These PGI_2s inhibited the transcellular transport dose-dependently. After the elimination of PGI_2, its inhibitory effect persisted for at least 1 hr. A good correlation was found between increase of cAMP and the potency of inhibition. Increase of cAMP after PGI_2 treatment seemed to be involved in the inhibition of FD transport.

The vascular endothelium has an important function as a selective barrier, such as in regulation of transport of macromolecules from the blood to the vessel wall [1, 2]. Further PGI_2,† a major product of arachidonic acid in endothelial cells [3, 4] is a potent inhibitor of platelet aggregation and adhesion [5, 6]. PGI_2 synthesis seems to be involved in the physiological mechanism for preventing thrombosis on the endothelium [7, 8], and thus in maintenance of smooth blood flow. In pathological conditions, such as inflammation, hypertension, hyperlipidemia and atherosclerosis, the endothelium tends to be injured [9, 10], with associated decrease in formation of PGI_2 [11, 12]. Decreased formation of PGI_2 may be one of the most important events in injury, but little is known about whether PGI_2 has any role in the transport of macromolecules through endothelial cells.

Previously we reported development of a model for study of intercellular junctional transport of FD [13]. This model enabled us to investigate the effect of PGI_2 on transport through the intercellular junctions. This paper reports studies using this model on the effects of stable PGI_2 deriv. (A) and (B) [14] and native PGI_2 on intercellular transport of FD.

MATERIALS AND METHODS

Prostaglandins and reagents. PGI_2 deriv. (A) and (B) were synthesized chemically at Teijin Co., Tokyo. PGI_2 and PGE_1 were purchased from Funakoshi Pharm. Co., Tokyo. Prostaglandins and their derivatives were dissolved in ethanol (5 mg/ml) and diluted with phosphate buffered saline before use. Dibutyryl cAMP (dbcAMP) and 3-isobutylmethylxanthine (IBMX) were purchased from Sigma Chemical Co. (St. Louis, MO). 2′,5′-Dideoxyadenosine (DDA) was from P.L. Biochem. (WI).

Preparation of a gelated collagen layer supported by a dacron sheet. Gelated collagen supported by a dacron sheet was prepared as described previously [15]. A circular piece of dacron sheet (2.4 cmϕ) was placed in a Falcon 3001 culture dish. Collagen solution (Koken Co., Tokyo: type I; 1.6 mg/0.8 ml/dish) was allowed to gel on the dacron sheet at room temperature and sterilized by u.v.-irradiation overnight.

Culture of arterial endothelial cells. Endothelial cells from porcine arterial wall were obtained by the method of Neichi *et al.* [16] with slight modification and cultured in Dulbecco's modified Eagle's minimum essential medium (DMEM; Flow Lab., VA) supplemented with 10% fetal calf serum (FCS; Flow Lab., VA). After 2 or 3 passages, cell suspensions (8×10^5/dish) treated with trypsin were seeded on the collagen gel. The confluent cell monolayers obtained by culture for 2 days were used for experiments.

Detection of FD transported through the endothelial cell layer. An endothelial cell monolayer on collagen gel supported by a dacron sheet was placed in the middle of a glass apparatus prepared in our laboratory [15]. The chamber was filled with DMEM supplemented with 10% FCS and placed in a 5% CO_2-incubator. FD (MW = 70,000; 20 μM; Sigma, MO) and a prostaglandin derivative were introduced into the upper chamber and the FD transported into the lower chamber was monitored with a fluorescence spectrophotometer (excitation, 495 nm, emission, 550 nm).

Detection of cAMP production. The reaction was

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† Abbreviations used: PGI_2, prostacyclin: FD, fluorescein dextran; cAMP, adenosine 3′,5′-cyclic monophosphate; IBMX, 3-isobutyl-methylxanthine, DDA, 2′,5′-dideoxyadenosine; dbcAMP, dibutyryl cAMP.

initiated by adding a prostaglandin derivative to the cell monolayer and terminated by adding cold trichloroacetic acid (TCA; final concentration; 5%). TCA was extracted from the acid-soluble fraction with water-saturated ether and cAMP was measured with a radioimmuno-assay kit (Amersham, Buckinghamshire).

RESULTS

1. Inhibitory effects of prostaglandins on FD transport

PGI_2 deriv. (A), isocarbacyclin, is a novel, stable derivative of PGI_2 (Fig. 1) [14]. The effect of PGI_2 deriv. (A) on transport of FD through endothelial cells with time was examined (Fig. 2). FD rapidly passed through a layer of collagen gel without an endothelial cell monolayer (880 pmol/cm^2/hr), but in the presence of endothelial cells, its passage was restricted (95 pmol/cm^2/hr). Incubation of endothelial cells with 3×10^{-6} M PGI_2 deriv. (A) resulted in decrease in their FD transport (47 pmol/cm^2/hr; 51% inhibition).

The effects of PGI_2 deriv. (A) and (B) and PGI_2 on the transport were measured at various times (Fig. 3). PGI_2 (A) and (B) caused about 45% and 33% inhibitions, respectively, for 4 hr, whereas the inhibition by PGI_2 gradually decreased after 2 hr. These results suggest that stable PGI_2 deriv. (A) and (B) inhibited the transport for longer than native PGI_2.

The potencies of various concentrations of prostaglandins were compared at the 2 hr rate (Fig. 4), because the effect of each prostaglandin did not change up to 2 hr (Fig. 3). Figure 4 shows that PGI_2 deriv. (A) and PGI_2 inhibited FD transport at concentrations of 3×10^{-9} M and that the minimum concentrations of these prostaglandins for maximal inhibitory effects were 3×10^{-8} M. The dose-dependency curves of PGI_2 deriv. (A) and PGI_2 were similar at 2 hr. The effect of PGI_2 deriv. (B) was

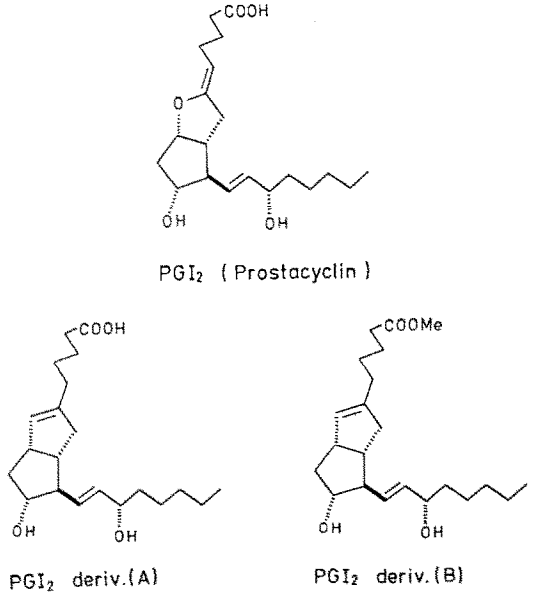

Fig. 1. Structures of PGI_2 and PGI_2 deriv. (A) and (B).

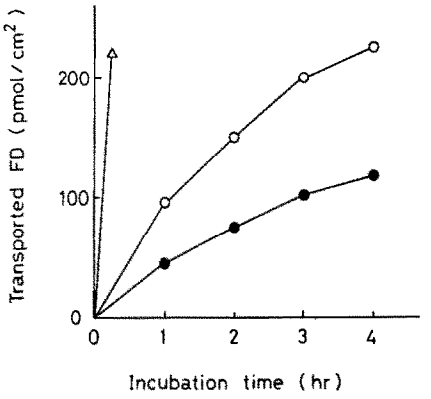

Fig. 2. Time course of FD transport. FD (20 μM, MW = 70,000) was added to the upper chamber and FD transported to the lower chamber (pmol/cm^2) was monitored. Results with a collagen gel layer (—△—), and a confluent endothelial cell layer on collagen gel in the absence (—○—) and presence (—●—) of 3 μM PGI_2 deriv. (A) are shown.

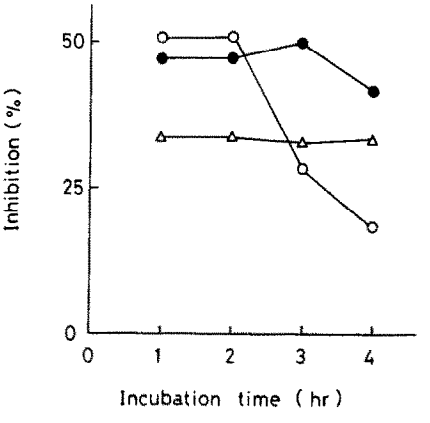

Fig. 3. Time courses of FD transport in the presence of PGs. The inhibitory effects (%) on FD transport of 3 μM PGI_2 (—○—), PGI_2 deriv. (A) (—●—) and PGI_2 deriv. (B) (—△—) are shown. Inhibitory effect (%) = [(FD in control) − (FD with PG)]/(FD in control).

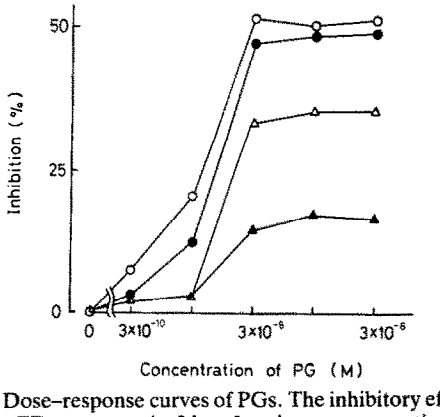

Fig. 4. Dose-response curves of PGs. The inhibitory effects (%) on FD transport in 2 hr of various concentrations of PGI_2 (—○—), PGI_2 deriv. (A) (—●—), PGI_2 deriv. (B) (—△—) and PGE_1 (—▲—) are shown.

Table 1. Persistence of inhibitory effect of PGI_2 on FD transport after its removal

	Transported FD (pmol/cm²)		
	0.5	1.0	2.0 (hr)
None	27.6 ± 5.1	73.4 ± 8.9	125.3 ± 9.8
PGI_2 deriv. (A) (not washed)	16.7 ± 2.6	48.9 ± 6.5	74.8 ± 6.5
PGI_2 deriv. (A) (washed)	13.8 ± 1.3	49.8 ± 7.2	71.7 ± 4.4
None	38.7 ± 5.2	72.9 ± 4.7	102.6 ± 7.3
PGI_2 (not washed)	17.4 ± 3.0	36.3 ± 4.3	45.6 ± 6.5
PGI_2 (washed)	21.6 ± 2.3	43.8 ± 6.4	60.3 ± 8.9

Endothelial cells were incubated with 3 μM PGI_2 or PGI_2 deriv. (A) for 10 min, and washed. FD transport (pmol/cm²) was measured in the absence (washed) or presence (not washed) of 3 μM PGI_2 or PGI_2 deriv. (A). Data are the means ± SD.

about three-fourths of that of PGI_2 deriv. (A) at 3×10^{-6} M. PGE_1 had the weakest inhibitory effect (about one-third that of PGI_2 deriv. (A)).

The effects of prostaglandins after their elimination from the medium were examined (Table 1). The inhibitory effect of PGI_2 deriv. (A) persisted for at least 2 hr. The half-life of PGI_2 is known to be a few minutes [17, 18], but its inhibitory effect persisted for up to 1 hr, suggesting that its inhibitory effect was indirect.

2. *Cyclic AMP and FD transport*

The inhibitory effects of these prostaglandins on FD transport may be connected with an intracellular mediator such as cAMP, because in general, cAMP increases after treatment with prostaglandins [19, 20]. This possibility was tested by measuring FD transport in the presence of reagents that are known to modulate the intracellular cAMP content (Table 2). IBMX (0.5 mM), which is known to increase the cAMP content by inhibiting cyclic nucleotide phosphodiesterase, inhibited FD transport by 26.0%. The inhibitory effect of PGI_2 deriv. (A) (42.2%) was enhanced in the presence of IBMX (60.2%). This inhibition was almost abolished (10.6%) by 0.1 mM DDA, which is reported to inhibit adenylate cyclase to decrease cAMP content [21]. Similar results were obtained with native PGI_2.

Table 2. FD transport in the presence of reagents that affect the cAMP content of cells

	FD (% inhibition)		FD (% inhibition)		FD (% inhibition)
None	76.3 ± 10.7	PGI_2 deriv. (A)	44.1 ± 5.9 (42.2)	PGI_2	42.5 ± 7.5 (44.3)
dbcAMP	49.8 ± 7.4 (34.7)	+IBMX	30.4 ± 6.0 (60.2)	+IBMX	23.2 ± 3.2 (69.6)
IBMX	56.5 ± 9.6 (26.0)	+DDA	70.9 ± 6.5 (7.1)	+DDA	64.3 ± 11.2 (15.7)
DDA	73.3 ± 13.3 (3.9)	+IBMX DDA	68.2 ± 9.3 (10.6)	+IBMX DDA	78.1 ± 8.6 (0)

FD transport (pmol/cm²) in 1 hr in the presence of test reagents (PG; 3 μM, dbcAMP: 0.1 mM, IBMX; 0.5 mM, DDA; 0.1 mM) was measured. Data are the means ± SD. Inhibitory effects (%) are shown in parentheses.

Table 3. Cyclic AMP contents in the presence of reagents that affect its production

	cAMP (pmol/10^6 cells)			
	None		IBMX	
	None	DDA	None	DDA
None	1.8 ± 0.2	1.3 ± 0.2	3.8 ± 0.6	1.0 ± 0.3
PGI_2	3.2 ± 0.6	1.3 ± 0.2	13.9 ± 0.3	3.0 ± 0.4
PGI_2 deriv. (A)	3.3 ± 0.5	1.3 ± 0.1	17.1 ± 0.5	3.0 ± 0.2
PGI_2 deriv. (B)	1.9 ± 0.1	1.6 ± 0.3	6.2 ± 0.3	2.3 ± 0.4
PGE_1	1.6 ± 0.3	1.2 ± 0.2	5.9 ± 0.7	1.9 ± 0.3

Cyclic AMP contents (pmol/10^6 cells) were measured after incubation of cells with 3 μM PG plus 0.5 mM IBMX and/or 0.1 mM DDA for 10 min. Data are the means ± SD.

The cAMP content in the presence of IBMX plus DDA was measured. The cAMP content was increased about 2-fold by incubation for 10 min in the presence of PGI_2 deriv. (A) and PGI_2 (Table 3). The increase was not so much in the case of PGI_2 deriv. (B) and PGE_1. In all cases these increases were markedly enhanced in the presence of IBMX: 4.5-fold of control with PGI_2 deriv. (A), 3.7-fold with PGI_2, 1.6-fold with PGI_2 deriv. (B) and 1.6-fold with PGE_1. Cyclic AMP formation accelerated by IBMX in the presence of prostaglandins was suppressed by DDA (control value 3.8 pmol/10^6 cells to 1.0; PGI_2 deriv. (A) 17.1 to 3.0; PGI_2 13.9 to 3.0; PGI_2 deriv. (B) 6.2 to 2.3; PGE_1 5.9 to 1.9). The effects of prostaglandins in increasing the level of cAMP correlated well with their inhibitory effects on FD transport through the endothelial cell monolayer.

DISCUSSION

Vascular integrity and function are mediated by the endothelial cell monolayer lining the vascular wall. These endothelial cells are thought to act as a barrier to various macromolecules [1, 2]. Once endothelial cells are injured, the accumulations of lipoproteins such as LDL increase [22, 23]. This appears to be a primary event in atherogenesis. The surface of the endothelial cells also acts as a protector of thrombosis, which stimulates permeability of the vascular wall. PGI_2, mainly produced in the endothelial cells, is one of the most potent known inhibitors of platelet aggregation [5–7]. But there are few reports on the direct effect of PGI_2 on the permeability of the endothelial cell barrier to macromolecules.

In this work, we found PGI_2 at 3×10^{-9} M caused 21% inhibition of transport of FD through an endothelial cell monolayer (Fig. 4). The inhibitory effect of the stable PGI_2 deriv. (A) persisted for at least 4 hr and that of native PGI_2 persisted for 2 hr (Fig. 3). PGI_2 deriv. (B) was less inhibitory and PGE_1 was the least inhibitory. Our finding that although the half-life of native PGI_2 is only a few minutes [17, 18], the inhibitory effect of PGI_2 was observed for at least 2 hr, suggested that a secondary mediator was involved in the inhibition. The results in Table 1 showing that inhibitory effect lasted for at least 1 hr after removal of PGI_2 support this interpretation.

One possible explanation of the inhibitory effects of prostaglandins is that the inhibition is due to their effects in increasing cAMP production as a secondary mediator [19, 20], because the cAMP content increased in the presence of PGI_2, which inhibited FD transport (Tables 2 and 3). To confirm the involvement of cAMP in this system, we tested the effects of some reagents that modulate production of cAMP. In the presence of IBMX, which is an inhibitor of cAMP phosphodiesterase, PGI_2 deriv. (A) increased the cAMP content from 3.3 pmol/10^6 cells to 17.1, in parallel with increase of its inhibition of FD transport from 42.2% in the absence, to 60.2% in the presence of IBMX (Tables 2 and 3). Addition of DDA, an inhibitor of adenylate cyclase, with PGI_2 deriv. (A) reduced the amount of cAMP from 17.1 pmol/10^6 cells to 3.0 and decreased the inhibitory effect of this prostaglandin derivative on FD transport from 60.2% to 10.6 (Tables 2 and 3). Connection between cAMP and FD transport was supported by the evidence obtained from forskolin, a direct activator of catalytic unit of adenylate cyclase [24, 25]. The production of cAMP was stimulated by 50 μM forskolin from 1.8 pmol/10^6 cells to 5.8 and FD transport was suppressed 41% in this condition. Similar results have been reported for the transport of Evans-blue through endothelial cells from the human umbilical vein in the presence of PGE_1 [26].

The inhibitory effects of PGI_2 on FD transport may be explained by increase of tight junctional connections between endothelial cells, because PGI_2 increased the electrical resistance between the apical and basal layer [2] from 18.4 $\Omega \cdot cm^2$ to 26.6 (data not shown). This interpretation is supported by evidence for a correlation between electrical resistance and the cAMP content of epithelial cells [27]. Cyclic AMP, which increases on PGI_2 treatment probably acts as a secondary mediator in stimulating production of tight junctions.

In some cases, such as inflammation, on the other hand, PGI_2 enhances capillary permeability [28]. The reason for the difference between findings in capillary and arterial endothelium is unknown, but it may be explained by difference in the origins and sources of the blood vessel.

This work showed that junctional transport of FD through aortic endothelial cells was inhibited by PGI_2 because of increased formation of tight junctions induced by either cAMP or PGI_2. Our results help in understanding the physiological and pharmacological effects of PGI_2 on barrier function in the endothelium.

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