AN IMPORTANT ROLE OF PROSTACYCLIN IN PORCINE THYROID CELLS IN CULTURE

Stimulation and refractoriness of cyclic AMP synthesis and iodine metabolism

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1. Introduction

The discovery of prostacyclin [1] opened a new area of prostaglandin research. PGI_2 is a potent stimulator of the adenylate cyclase–cyclic AMP (cAMP) system [2,3] and is considered to be an important regulator of cell metabolism. We have shown that cultured porcine thyroid cells produce PGI_2 [4]. This raised a possibility that PGI_2 plays an important role in the thyroid physiology.

To test this hypothesis, we first studied the effects of PGI_2 on cAMP synthesis and iodine metabolism, using cultured porcine thyroid cells. We then attempted to study whether pre-exposure to PGE_1 or PGE_2 could make the thyroid cells refractory to further stimulation of PGI_2 on cAMP synthesis and iodine metabolism. The results show that PGI_2 stimulates cAMP synthesis and iodine metabolism and that pre-exposure to PGE_1 or PGE_2 induces refractoriness to further PGI_2-stimulation.

2. Materials

2.1. *Thyroid cell*

Thyroid cells were isolated from porcine thyroid glands of adult animals by a discontinuous trypsinization technique [5]. Freshly isolated cells, suspended in Eagle's minimum essential medium (pH 7.4) with 10% (v/v) calf serum, penicillin (200 units/ml) and streptomycin sulfate (50 μg/ml) were incubated at 3×10^6 cells/ml in Falcon plastic Petri dishes not treated for tissue culture. Cells were incubated at 37°C in 95% air–5% CO_2.

2.2. *Cell washing*

After 6 days' incubation, cells were centrifuged at $400 \times g$ for 5 min at 4°C, the supernatant was discarded and the pellet was resuspended in a phosphate-buffered saline (pH 7.4) (PBS) of the following composition (in mg/l): NaCl 8000, KCl 200, $Na_2HPO_4 \cdot 2\,H_2O$ 2890, KH_2PO_4 200, $CaCl_2 \cdot 2\,H_2O$ 66.6 and $MgCl_2 \cdot 6\,H_2O$ 100. Cells were centrifuged again as before. This washing procedure was repeated 3 times. After the last washing, the cells were suspended in phosphate-buffered saline containing 0.1% glucose (PBSG).

2.3. *Cyclic AMP assay*

Aliquots (120 μl) of the washed thyroid cell suspensions were incubated in air for 5 min at 37°C in a final volume of 150 μl containing PBSG, 10 mM theophylline and prostaglandins (PGs: PGE_1, PGE_2 and PGI_2). The incubation was ended by immersing the tubes into a dry ice–acetone bath until frozen, followed by subsequent immersion in a boiling water bath for 2 min. The cells were homogenized and centrifuged. Aliquots of the supernatants were diluted appropriately and used for assay of cAMP by radioimmunoassay as in [6].

2.4. *Iodine metabolism*

After 6 days' incubation, iodide uptake, discharge and organification of iodine were estimated using the washed cell supensions. Iodide uptake was measured as follows: aliquots (400 μl) of the cell suspensions were added to iodide solution (100 μl) to make a final volume of 500 μl containing 0.5 μM $Na^{127}I$ and 0.1 μCi $Na^{125}I$ in the presence of 1 mM methylmercaptoimidazole (MMI). After 10–40 min incubation in air at 37°C, 5 ml precooled (0°C) PBS was rapidly

added to stop iodide uptake, and the tubes were centrifuged at 1500 × *g* for 3 min. The supernatants were aspirated and the cell pellets were washed twice with PBS. The radioactivity levels of the washed cell pellets were measured in a well-type scintillation counter to indicate iodide uptake.

Measurement of iodide discharge was performed after loading the cells with iodide. Aliquots of the cell suspensions were incubated in the presence of 1 mM MMI in air for 30 min in a final volume of 500 μl PBSG containing $Na^{127}I$ (0.5 μM, final) and 0.1 μCi $Na^{125}I$. After 30 min incubation, 50 μl of solution of prostaglandins (PGs) was added and then the cell suspensions were incubated for 10 min unless otherwise stated. This 10 min incubation was ended by adding 5 ml precooled PBS and washing was performed twice with the same buffer as described above. The radioactivity levels of the cell pellets were counted.

To measure organification of iodine, aliquots of the cell suspensions were incubated with iodide solution in the absence of MMI in air for 30–60 min in a final volume of 500 μl PBSG containing 0.5 μM $Na^{127}I$ and 0.1 μCi $Na^{125}I$. After 30–60 min incubation, 3 ml 10% trichloroacetic acid was added to stop iodine organification and then the tubes were centrifuged at 1500 × *g* for 3 min. The cell pellets were then washed 3 times with trichloroacetic acid and trichloroacetic acid-insoluble radioactivity levels were counted.

To measure acute stimulatory action of PGs on organification of iodine, aliquots of the cell suspensions were incubated with iodide solution (0.5 μM $Na^{127}I$ and 0.1 μCi $Na^{125}I$) in the absence of MMI in air for 30 min in a final volume of 500 μl PBSG. After 30 min incubation, 50 μl PGs solution was added, and the cell suspensions were incubated for further 30 min. The incubation was ended by adding trichloroacetic acid, and the incubation tubes were centrifuged at 1500 × *g* for 3 min. The cell pellets were washed 3 times with trichloroacetic acid and trichloroacetic acid-insoluble radioactivity levels were counted.

The absolute amounts of iodine uptake, organification and discharge were calculated from the specific radioactivities of the original iodide solution. The amount of iodine was expressed as the mean or mean ± SE of triplicate determinations.

2.5. *Materials*

Thyroid stimulating hormone (TSH) was obtained from Armour Pharmaceuticals (Phoenix AZ). PGE_1, PGE_2 and PGI_2 were kindly donated by Ono Pharmaceuticals (Ohsaka). Purchases were made from the following sources: trypsin from Grand Island Biochemical (Grand Island NY); new born calf serum and basal medium Eagle from Flow Labs. (Irvine); $Na^{125}I$ from New England Nuclear. All other chemicals were of the highest purity available commercially.

3. Results

3.1. *Acute effects of graded doses of PGI_2 and PGE_2 on cAMP synthesis*

Isolated porcine thyroid cells were cultured in the presence of 0.05 mU/ml TSH. This concentration of TSH maintained the thyroid cells in the best condition and did not induce refractoriness to further stimulation [7–9]. After 6 days' incubation, the cells were washed and again incubated in the presence of 10 mM theophylline in room air for 5 min (fig.1A). After this 5 min incubation, cAMP concentrations were measured and these cAMP concentrations increased progressively with the increases of PGI_2 and PGE_2 doses; 0.01 μM PGI_2 and PGE_2 stimulated cAMP synthesis slightly and the maximal responses were obtained with 10 μM PGI_2 as far as examined. The cAMP response with 10 μM PGI_2 was ~1/2 of that with 10 μM PGE_2 and the cAMP response with 10 μM PGE_1 was the same as that with 10 μM PGE_2.

3.2. *Induction of refractoriness to PGI_2-, PGE_1- and PGE_2-stimulation of cAMP synthesis by previous exposure to PGE_1 and PGE_2*

Cells were cultured in the presence of 0.05 mU/ml TSH for 5 days, then exposed to 10 μM PGE_2 (fig.1B) or PGE_1 (fig.1C) for 24 h in the culture condition. After the 24 h incubation, the cells were washed and then incubated with graded doses of PGI_2, PGE_2 or 10 μM PGE_1 in the presence of 10 mM theophylline in room air for 5 min. After 5 min incubation, the cAMP concentrations were measured (fig.1B,C). PGE_2 and PGI_2 augmented the cAMP concentrations progressively with the increases of the doses as was observed in the cells not exposed to PGs (fig.1A). However, the magnitudes of cAMP responses were greatly reduced after exposure to 10 μM PGE_2 or PGE_1 (fig.1A–C), indicating the induction of refractoriness of PGI_2-, PGE_2- and PGE_1-stimulated cAMP synthesis after exposure to PGE_2 or PGE_1.

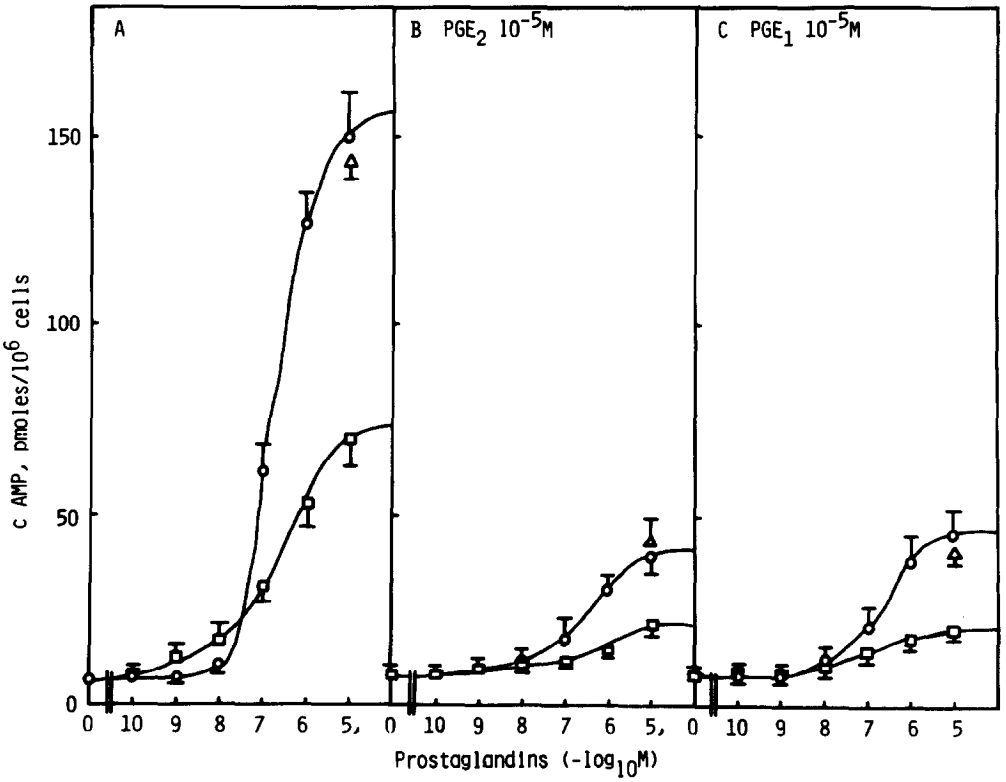

Fig.1. Acute effects of 10 μM PGE_1 (△) and graded doses of PGE_2 (○) and PGI_2 (□) on cAMP synthesis and the effects of 24 h exposure to PGE_2 (B) or PGE_1 (C) on PGE_1 (△), PGE_2 (○) and PGI_2 (□) -stimulated cAMP synthesis. The cells, incubated in the presence of 0.05 mU/ml TSH for 6 days (A), and the cells, incubated in the presence of 0.05 mU/ml for 5 days and then exposed to 10 μM PGE_2 (B) or PGE_1 (C) for 24 h, were washed and the washed cells again incubated with 10 μM PGE_1 (△) or graded doses of PGE_2 (○) or PGI_2 (□) for 5 min in the presence of 10 mM theophylline. The cAMP concentrations after the final 5 min incubation are shown. Each point is the mean ± SE of triplicate determinations.

3.3. *Discharge of accumulated iodide from the thyroid cells by PGI_2 and PGE_2*

When thyroid cells were cultured in the presence of 0.05 mU TSH/ml for 6 days and then incubated with 0.5 μM $Na^{127}I$ and 0.1 μCi $Na^{125}I$ in the presence of 1 mM MMI, the cells took up iodide. As shown in fig.2A, the uptake was maximum at 30 min. After 30 min incubation with iodide, 10 μM PGI_2 or PGE_2 was added and these PGs caused a rapid discharge of intracellular iodide. This iodide discharge was observed within 2 min and the maximal iodide discharge was observed around 10 min after adding PGs. The absolute amounts of iodide discharge (fig.2A, closed symbols) were the differences of iodide concentrations between control and PGs-added cells. The magnitude of this iodide discharge depended on the PGs concentrations (fig.2B); 0.01 μM PGE_2 and PGI_2 caused a slight discharge of iodide and maximal iodide discharge was observed when 10 μM PGE_2 or PGI_2 was administered as an acute stimulator. The amount of iodide discharge produced by 10 μM PGI_2 was ~2/3rd, that produced by 10 μM PGE_2.

3.4. *Induction of refractoriness to PGI_2-, PGE_1- and PGE_2-stimulation of iodide discharge by previous exposure to PGE_1 or PGE_2*

The effects of pre-exposure to PGE_2 (fig.2C) or PGE_1 (fig.2D) on PGI_2-, PGE_2- and PGE_1-stimulated iodide discharge were studied. Cells were cultured in the presence of 0.05 mU/ml TSH for 5 days and then 10 μM PGE_2 (fig.2C) or PGE_1 (fig.2D) was added to the medium and the incubation was continued for further 24 h. After this incubation, the cells were washed and then incubated with 0.5 μM $Na^{127}I$ and 0.1 μCi $Na^{125}I$ for 30 min in the presence of MMI.

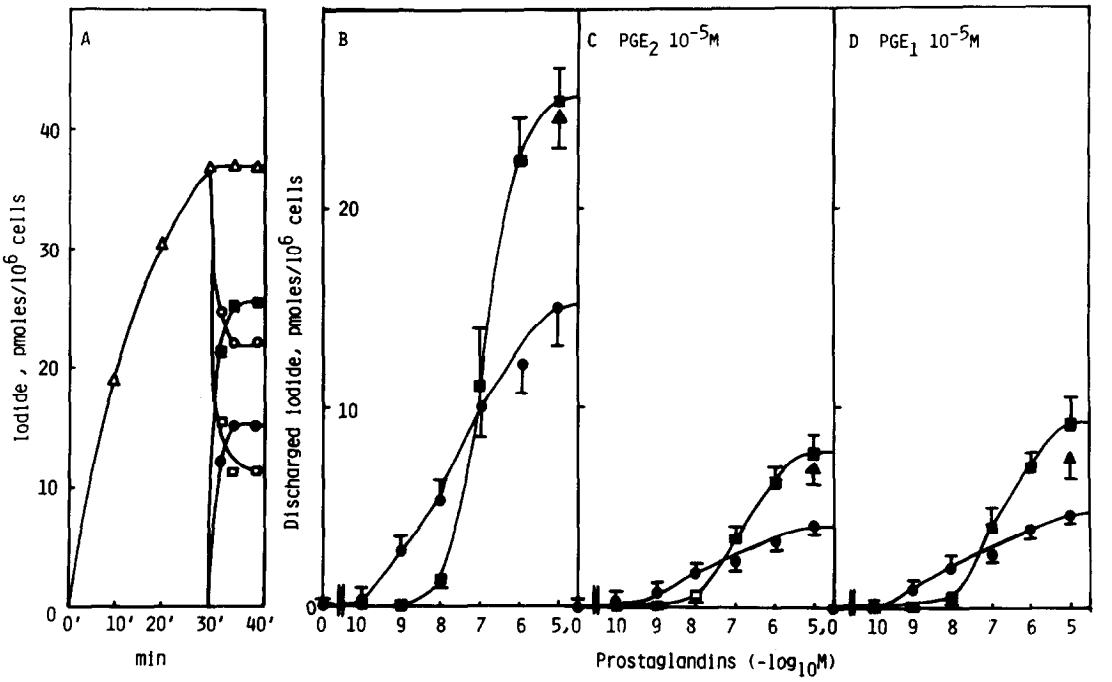

Fig.2. Iodide discharge induced by PGs and the effects of 24 h exposure to PGE_2 (C) and PGE_1 (D) on PGE_1 (▲), PGE_2 (■) and PGI_2 (●) -stimulated iodide discharge. (A) The cells, incubated in the presence of 0.05 mU TSH/ml were washed, then incubated with 0.5 μM $Na^{127}I$ and 0.1 μCi $Na^{125}I$ (NaI) in the presence of 1 mM MMI. The cells took up iodide (△). After 30 min incubation with NaI, 10 μM PGI_2 (○), PGE_2 (□) or buffer (△) was added, then the incubation was continued further for the indicated periods. The differences of iodide concentrations between PGs-added (PGE_2 (□); PGI_2 (○)) and buffer-added (△) ones indicated the amounts of iodide discharge (PGE_2 (■); PGI_2 (●)). (B) The cells, incubated in the presence of 0.05 mU/ml TSH for 6 days, were washed and then incubated with NaI in the presence of MMI. After 30 min incubation with NaI, 10 μM PGE_1 and graded doses of PGE_2 and PGI_2 were added and the incubation was continued further for 10 min to get acute effects of PGs on iodide discharge. The absolute amounts of iodide discharge by PGE_1 (▲), PGE_2 (■) and PGI_2 (●) were calculated as in (A). (C,D) The cells, incubated in the presence of 0.05 mU TSH/ml for 5 days and then exposed to 10 μM PGE_2 (C) or PGE_1 (D) for 24 h, were washed, then incubated with NaI in the presence of MMI. After 30 min incubation with NaI, 10 μM PGE_1 and graded doses of PGE_2 and PGI_2 were added and then the incubation was continued further for 10 min to get acute effects of PGs on iodide discharge. The absolute amounts of iodide discharge by PGE_1 (▲), PGE_2 (■) and PGI_2 (●) were calculated as in (A). Each point is the mean or mean ± SE of triplicate determinations.

After attaining equilibrium with 30 min incubation with NaI, graded doses of PGI_2, PGE_2 and 10 μM PGE_1 were added. Exposure of 24 h to 10 μM PGE_2 or PGE_1 lessened the degrees of PGI_2-, PGE_2- and PGE_1-stimulated iodide discharge; the degree of PGI_2-, PGE_2- or PGE_1-stimulated iodide discharge after exposure to 10 μM PGE_2 or PGE_1 was ~1/3rd of that not exposed to the PGs.

3.5. *PGI_2- and PGE_2-stimulated iodide organification*

Acute effects of PGI_2 and PGE_2 on iodine organification were studied. The cells cultured in the presence of 0.05 mU/ml TSH for 6 days were washed and then incubated with 0.5 μM $Na^{127}I$ and 0.1 μCi $Na^{125}I$ in the absence of MMI. As shown in fig.3A, iodine organification increased progressively with time. After 30 min incubation, 10 μM PGI_2 or PGE_2 was added and these stimulators increased total amounts of organic iodine (fig.3A). The stimulation was apparent when the absolute increment of organic iodine was calculated (fig.3A, closed symbols).

The magnitude of this iodine organification depended on the PGs concentrations (fig.3B); 0.01 μM PGE_2 and PGI_2 caused a slight increase of iodine organification and maximal iodine organification was observed when 10 μM PGE_2 or PGI_2 was administered as an acute stimulator. The amount of iodine organification produced by 10 μM PGI_2 was ~2/3rds that produced by 10 μM PGE_2.

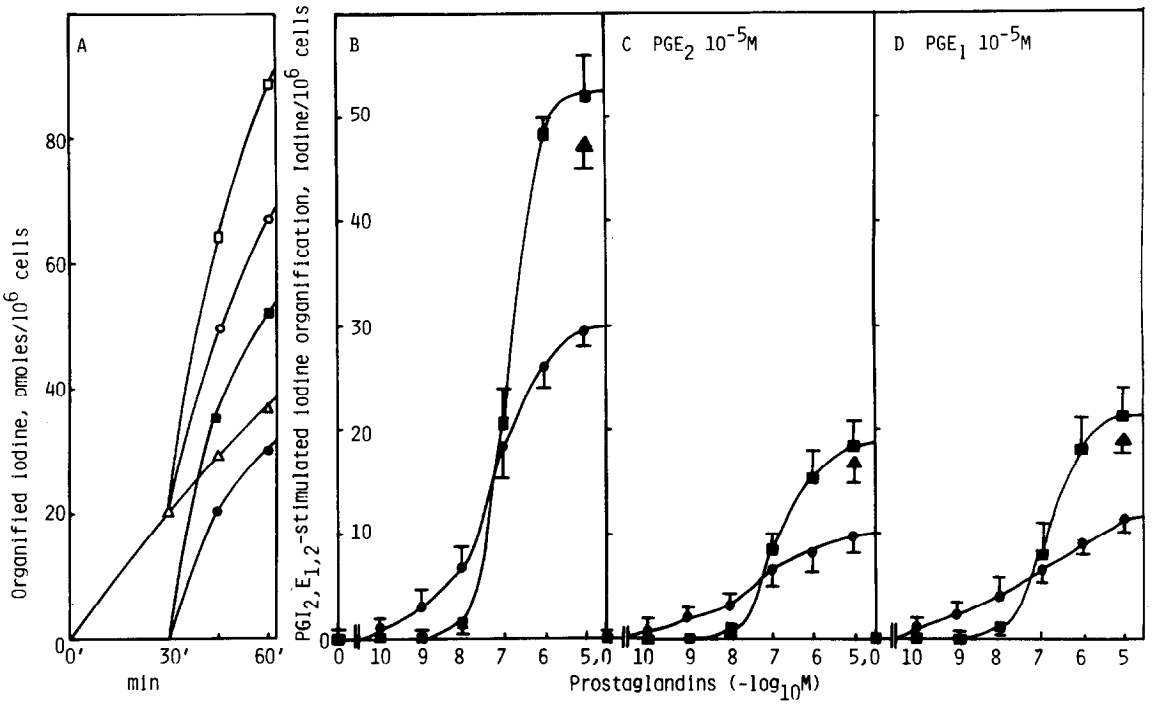

Fig.3. PGs-stimulated iodine organification and the effects of 24 h exposure to PGE_2 (C) and PGE_1 (D) on PGE_1 (▲), PGE_2 (■) and PGI_2 (●) -stimulated iodine organification. (A) Thyroid cells, incubated in the presence of 0.05 mU TSH/ml, were washed, then incubated with 0.05 μM $Na^{127}I$ and 0.1 μCi $Na^{125}I$ (NaI) in the absence of MMI. After 30–60 min incubation, iodine organification (trichloroacetic acid-insoluble radioactivity) was measured (△). After 30 min incubation, 10 μM PGE_2 (□), PGI_2 (○) or buffer (△) was added, then the incubation continued for further 15–30 min. The differences of iodine organifications between PGs-added (PGE_2 (□); PGI_2 (○)) and buffer-added (△) ones indicated the amounts of PGE_2 (■) or PGI_2 (●) -stimulated iodine organification, respectively. (B) The cells, incubated in the presence of 0.05 mU TSH/ml for 6 days, were washed and then incubated with NaI. After 30 min incubation with NaI, 10 μM PGE_1 and graded doses of PGE_2 and PGI_2 were added and then the incubation was continued for 30 min to get acute effects of PGE_1, PGE_2 and PGI_2 on iodine organification. The amounts of PGE_1 (▲), PGE_2 (■) and PGI_2 (●) -stimulated iodine organification were calculated as in (A). (C,D) The cells, incubated in the presence of 0.05 mU TSH/ml for 5 days, then exposed to 10 μM PGE_2 (C) or PGE_1 (D) for 24 h, were washed, then incubated with NaI. After 30 min incubation with NaI, 10 μM PGE_1 and graded doses of PGE_2 and PGI_2 were added, then the incubation was continued further for 30 min to get acute effects of PGE_1, PGE_2 and PGI_2 on iodine organification. The amounts of PGE_1 (▲), PGE_2 (■) and PGI_2 (●) -stimulated iodine organification were calculated as in (A). Each point is the mean or mean ± SE of triplicate determinations.

3.6. *Induction of refractoriness to PGI_2-, PGE_1- and PGE_2-stimulation of iodine organification by previous exposure to PGE_1 and PGE_2*

The effects of pre-exposure to PGE_2 and PGE_1 on PGI_2-, PGE_2- and PGE_1-stimulated iodine organification were studied. Cells were cultured in the presence of 0.05 mU TSH/ml for 5 days and then 10 μM PGE_2 (fig.3C), or PGE_1 (fig.3D) was added to the medium and the incubation was continued for further 24 h. After this incubation, the cells were washed and then incubated with 0.5 μM $Na^{127}I$ and 0.1 μCi $Na^{125}I$. After 30 min incubation with NaI, graded doses of PGI_2, PGE_2 or 10 μM PGE_1 were added and then the incubation was continued for further 30 min and the absolute increment of organic iodine was calculated. Exposure of 24 h to 10 μM PGE_2 (fig.3C), or PGE_1 (fig.3D) lessened the degrees of PGs-stimulated iodine organification. The degrees of PGs-stimulated iodine organification after exposure to PGs were ~1/3rd of those not exposed to the PGs (fig.3B–D).

4. Discussion

Cultured porcine thyroid cells synthesize PGI_2 [4] and PGI_2 was reported to be a potent stimulator of

adenylated cyclase–cAMP synthesis [2,3]. Thus it seems important to evaluate PGI_2 as a potential stimulator of cAMP synthesis and iodine metabolism in the thyroid and this study shows a number of important effects of PGI_2 on the thyroid cells in culture.

As expected, PGI_2 stimulates cAMP synthesis of the thyroid cells. Cyclic AMP has been known to play an important regulatory role on iodine metabolism [10] and PGI_2 was expected to affect iodine metabolism through cAMP production. As expected, PGI_2 stimulates iodine discharge and organification of the thyroid cells in culture. Thus PGI_2 stimulates cAMP synthesis, iodine discharge and organification. The cultured porcine thyroid cells produce PGI_2 in addition to PGE_2 and $PGF_{2\alpha}$ and in the presence of thyroid stimulating hormone (TSH), PGI_2 plays a more important role than other prostaglandins [4]; the concentrations of PGI_2 of the cells cultured in the presence of TSH are higher than those of the cells cultured in the absence of TSH but the concentrations of PGE_2 and $PGF_{2\alpha}$ of the cells cultured in the presence of TSH are much lower than those of the cells cultured in the absence of TSH. These findings provide evidence that PGI_2 plays an important role in thyroid physiology.

As a next step, the refractory process was studied to reveal the functional interaction among the prostaglandins: PGI_2, PGE_1 and PGE_2. Pre-exposure to PGE_2 makes the thyroid cells refractory to further PGE_1- and PGI_2-stimulation in addition to PGE_2-stimulation of cAMP synthesis [8]. Previous exposure to PGE_1 also makes the thyroid cells refractory to further PGE_2- and PGI_2-stimulation in addition to PGE_1-stimulation. These results indicate that pre-exposure to one prostaglandin induced refractoriness to further prostaglandins stimulation, suggesting the existence of an interaction of one prostaglandin with another prostaglandin-stimulated cAMP synthetic process. Refractoriness was observed at the level of the cAMP synthesis and cAMP is an important regulator of iodine metabolism. Thus refractoriness of iodine metabolism was studied and, as expected, similar type of refractoriness at the level of iodine metabolism is clearly observed; pre-exposure to PGE_2 or PGE_1 induces refractoriness to further PGI_2-, PGE_2- and PGE_1-stimulated iodine discharge and organification.

PGI_2 stimulates cAMP synthesis and iodine metabolism. These PGI_2-stimulated cAMP synthesis and iodine metabolism are modulated by the pre-exposure to prostaglandins. Thus PGI_2 plays an important role in thyroid physiology and PGI_2 will provide a new tool to study receptor adenylate cyclase–cAMP system, refractoriness, iodine metabolism and the mechanism of the roles of prostaglandins in the thyroid gland.

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