AUGMENTATION OF PROSTACYCLIN AND DEPRESSION OF PGE_2, $PGF_{2\alpha}$ AND THROMBOXANE A_2 BY TSH IN CULTURED PORCINE THYROID CELLS

An important role of prostacyclin in maintaining thyroid cell function

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1. Introduction

The importance of prostaglandins (PGs) in thyroid physiology has long been a controversial subject. In [1–3] 6-ketoprostaglandin $F_{1\alpha}$ was isolated and found to be an end-metabolite of the unstable compound, prostacyclin [4,5]. Prostacyclin producing activity has been reported in many tissues and cells [6–8], and biological significance of prostacyclin in maintaining homeostasis has become apparent. Thromboxane B_2, an end-metabolite of thromboxane A_2, has been isolated [9] and this thromboxane A_2 seems to play some role to maintain homeostasis. However, prostacyclin and thromboxane A_2 producing activities in the thyroid have not been reported and their biological significance needs to be studied.

To evaluate the role of endogenous PGs in the regulation of thyroid function, we have estimated prostaglandin E_2 (PGE_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), 6-ketoprostaglandin $F_{1\alpha}$ and thromboxane B_2 (TXB_2) and correlations of these PGs and thyroid functions were studied using cultured porcine thyroid cells. Here, we show that in the absence of TSH, the cells are unable to take up iodide or organify it but produce PGE_2, $PGF_{2\alpha}$ and TXB_2 and that in the presence of TSH, the cells are able to take up iodide and organify it but preferentially produce 6-ketoprostaglandin $F_{1\alpha}$, an end-metabolite of prostacyclin.

2. Materials and methods

2.1. *Cell culture*

Thyroid cells were obtained from porcine thyroid gland by a discontinuous trypsinization procedure [10]. Freshly isolated cells were suspended (3×10^6 cells/ml) in Eagle minimum essential medium supplemented with 10% newborn calf serum (Flow Labs.) and antibiotics (penicillin, 200 units/ml, streptomycin, 50 μg/ml). They were incubated as unstirred suspensions in polystyrene Petri dishes not treated for tissue culture at 37°C in a 5% CO_2–95% air, water-saturated atmosphere.

2.2. *Prostaglandin measurement*

After 5 days culture, cells were collected by centrifugation at 200 × *g* for 5 min at 4°C. The resulting pellets were suspended in 1 ml 10 mM phosphate buffer (pH 7.2) and homogenized. Cell homogenate and medium were processed as follows. PGE_2 and $PGF_{2\alpha}$ were measured according to [11] as in [12]. Tracer amounts of labeled PGE_2 and $PGF_{2\alpha}$ were added to evaluate the yield of the extraction process. Briefly, the homogenate and the medium were mixed with 3 ml petroleum ether and neutral fat was removed. The aqueous layer was then well mixed with 3 ml mixture of ethyl acetate, isopropanol and 0.2 N HCl (3:3:1, by vol.), and 2 ml ethyl acetate and 3 ml distilled water were further added. This mixture was centrifuged at 1100 × *g* for 5 min. Organic phase (3 ml) was removed and evaporated to dryness at a reduced pressure. The dried material was finally dissolved in

1 ml 50 mM Tris–HCl buffer (pH 7.4) with 0.03% gelatin. The dissolved material was applied to a silicic acid column to isolate PGE_2 and $PGF_{2\alpha}$. Concentrations of the isolated PGE_2 and $PGF_{2\alpha}$ were measured by radioimmunoassay (Clinical Assay, Cambridge MA). 6-Ketoprostaglandin $F_{1\alpha}$ and TXB_2 were measured according to [13] (in preparation). Briefly, tracer amounts of labeled 6-ketoprostaglandin $F_{1\alpha}$ and TXB_2 were added to evaluate the yield of the extraction process. The homogenate and the medium were mixed with chloroform/methanol (2:1, v/v) mixture. After passing through Sephadex G-25 column, the eluates were mixed with carbon tetrachloride/phosphate buffer containing 10% methanol (1:3, v/v). The phosphate buffer phase, containing 6-ketoprostaglandin $F_{1\alpha}$ and TXB_2, was collected and acidified to pH 3 with 2 N HCl and then ethyl acetate was added. The ethyl acetate phase was collected, neutralized, dried and then applied to thin-layer chromatography (Silica gel, 60HR, Merck). Analysis was done using a solvent system of ethyl acetate/chloroform/methanol/acetic acid (200:200:40:10, by vol.). Spots corresponding to 6-ketoprostaglandin $F_{1\alpha}$ and TXB_2 were scraped off and extracted with methanol containing 0.5% acetic acid. Concentrations of the extracted 6-ketoprostaglandin $F_{1\alpha}$ and TXB_2 were measured by radioimmunoassay [13] (Ono Pharmaceutical Co., Ohsaka).

2.3. *Iodine metabolism*

Iodine uptake and organification were estimated as in [14]. After 5 days' culture, the cells were washed 3 times with phosphate-buffered saline (pH 7.4) (PBS, 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; $MgCl_2 \cdot 6\,H_2O$ 100; then suspended in PBS with 0.1% glucose (PBSG). Aliquots (400 μl) of the cell suspension were added to iodide solution (100 μl) to make 500 μl final vol. containing 0.5 μM $Na^{127}I$ and 0.1 μCi $Na^{125}I$ (final conc.). After 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. To express organic iodine, the cell pellets were washed 3 times with 10% trichloroacetic acid and trichloroacetic acid-insoluble radioactivity levels were measured.

2.4. *Materials*

TSH was obtained from Armour Pharmaceutical Co. (Phoenix AZ). PGE_2 and $PGF_{2\alpha}$ were kindly donated by Ono Phamaceutical Co. (Ohsaka). Purchases were made from the following sources: trypsin from Grand Island Biological Company (Grand Island NY); new-born calf serum and basal medium Eagle from Flow Labs. (Irvine, Scotland); $Na^{125}I$ from New England Nuclear. All other chemicals were of the highest purity available commercially.

3. Results

3.1. *Effect of chronic exposure to graded doses of TSH on PGE_2, $PGF_{2\alpha}$, TXB_2 and 6-ketoprostaglandin $F_{1\alpha}$*

Porcine thyroid cells were cultured in the presence of graded doses of TSH (0–50 mU/ml) for 5 days, then PGE_2 (fig.1), $PGF_{2\alpha}$ (fig.2), TXB_2 and 6-keto-

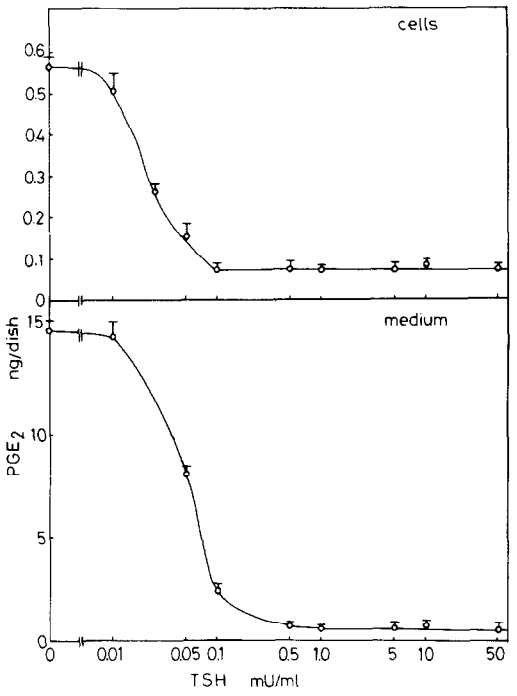

Fig.1. Effect of 5 days' exposure to graded doses of TSH on PGE_2 accumulation in the cells and its release into the culture medium. Isolated porcine thyroid cells were cultured in the presence of graded doses of TSH for 5 days, then PGE_2 contents of the cells and the medium were measured. One dish contains 9 × 10^6 cells or 3 ml cell suspension. Each point is the mean ± SE of 3 determinations.

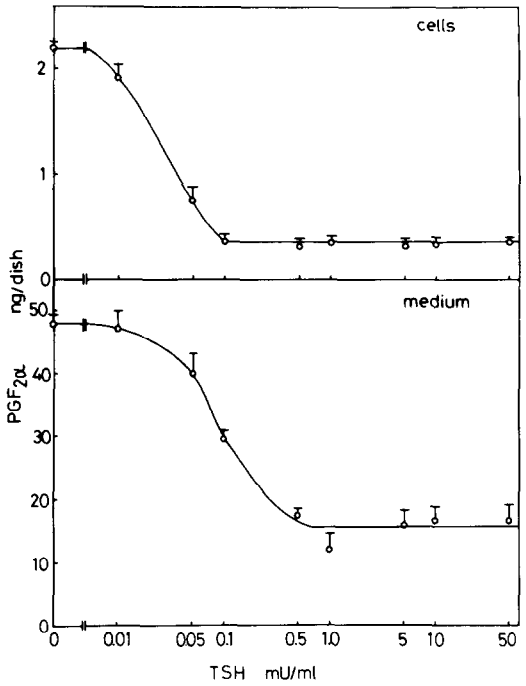

Fig.2. Effect of 5 days' exposure to graded doses of TSH on $PGF_{2\alpha}$ accumulation in the cells and its release into the culture medium. Isolated porcine thyroid cells were cultured in the presence of graded doses of TSH for 5 days and then $PGF_{2\alpha}$ contents of the cells and the medium were measured. One dish contains 9×10^6 cells or 3 ml cell suspension. Each point is the mean ± SE of 3 determinations.

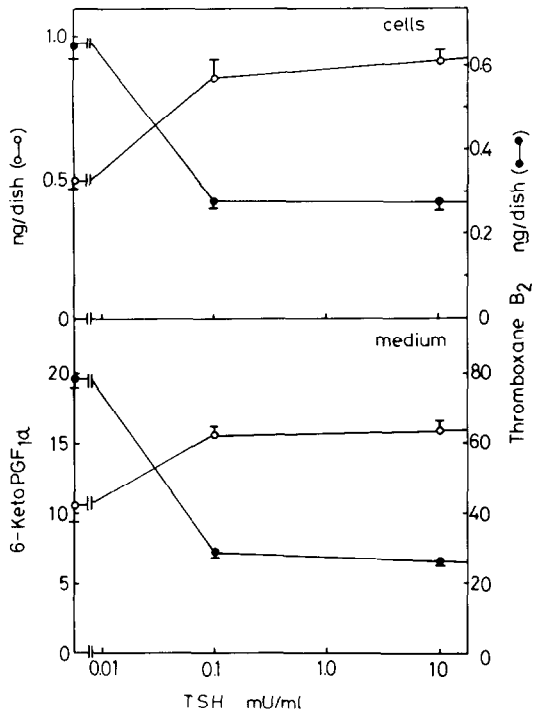

Fig.3. Effect of 5 days' exposure to graded doses of TSH on 6-ketoprostaglandin $F_{1\alpha}$ and TXB_2 accumulation in the cells and their release into the culture medium. Isolated porcine thyroid cells were cultured in the presence of graded doses of TSH for 5 days, then 6-ketoprostaglandin $F_{1\alpha}$ and TXB_2 contents of the cells and the medium were measured. One dish contains 9×10^6 cells or 3 ml cell suspension. Each point is the mean ± SE of 3 determinations.

prostaglandin $F_{1\alpha}$ (fig.3) contents in the cells and medium were measured. When the cells were cultured in the absence of TSH, PGE_2, $PGF_{2\alpha}$ and TXB_2 contents were high but when the cells were cultured in the presence of TSH, they were low. A decrease of these protaglandin contents was inversely correlated with the doses of TSH in the culture medium: slight decreases of PGE_2 and $PGF_{2\alpha}$ were observed when the cells were cultured in the presence of 0.01 mU/ml TSH and maximal decreases of PGE_2 and $PGF_{2\alpha}$ were observed when the cells were cultured in the presence of 0.1 mU/ml TSH (fig.1,2, cells) or 0.5 mU/ml TSH (fig.1,2, medium). When the cells were cultured in the presence of higher concentrations of TSH (>0.5 mU/ml), no further reductions of PGE_2, $PGF_{2\alpha}$ and TXB_2 were observed. PGE_2, $PGF_{2\alpha}$ and TXB_2 contents in the medium were greater than those in the cells. In the absence of TSH, the concentrations of $PGF_{2\alpha}$ and TXB_2 were ~3 or 5 times more than that of PGE_2, respectively.

In sharp contrast, concentrations of 6-ketoprostaglandin $F_{1\alpha}$ in the cells and medium increased when the cells were cultured in the presence of 0.1 mU/ml TSH. No further increase of 6-ketoprostaglandin $F_{1\alpha}$ was observed when the cells were cultured in the presence of 10 mU/ml TSH. Absolute amount of 6-ketoprostaglandin $F_{1\alpha}$ was greater in the medium than in the cells.

3.2. *Effect of chronic exposure to graded doses of TSH, PGE_2 and $PGF_{2\alpha}$ on iodine uptake and organification*

When thyroid cells were cultured in the absence of TSH, no significant uptake and organification of iodine were observed. Thyroidal uptake and organification of iodine were augmented when the cells were cultured in the presence of TSH (fig.4). The magnitude of augmentation was dependent on [TSH] in the

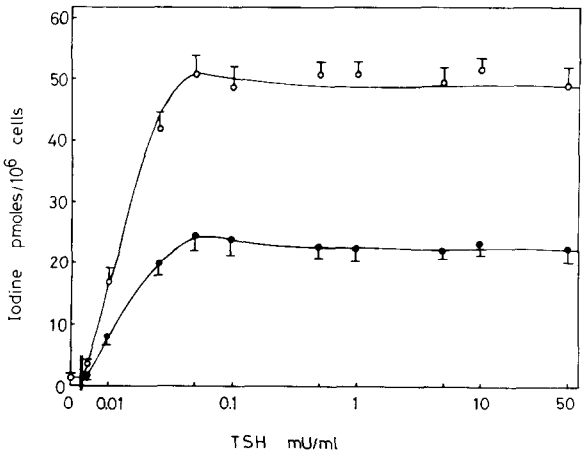

Fig.4. Effect of 5 days' exposure to graded doses of TSH on iodine uptake and organification. Isolated porcine thyroid cells were cultured in the presence of graded doses of TSH and then the cells were incubated with 0.5 μM $Na^{127}I$ and 0.1 μCi $Na^{125}I$. After 40 min incubation, iodide uptake (○) was measured then iodine organification (trichloroacetic acid-insoluble radioactivities (●)) was measured. Each point is the mean ± SE of 3 determinations.

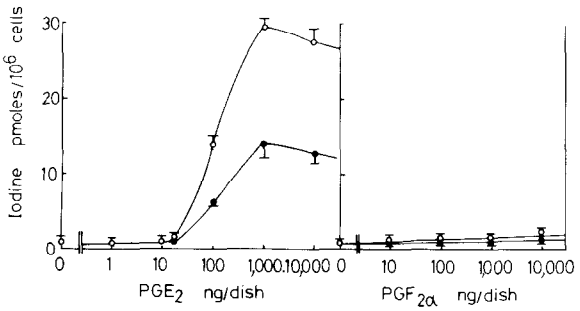

Fig.5. Effect of 5 days' exposure to graded doses of PGE_2 and $PGF_{2\alpha}$ on iodine uptake and organification. Isolated porcine thyroid cells were cultured in the presence of graded doses of PGE_2 and $PGF_{2\alpha}$ and then the cells were incubated with 0.5 μM $Na^{127}I$ and 0.1 μCi $Na^{125}I$. After 40 min incubation, iodide uptake (○) was measured and then iodine organification (trichloroacetic acid-insoluble radioactivities (●)) was measured. Each point is the mean ± SE of 3 determinations.

culture medium: at 0–0.05 mU/ml iodine uptake and organification were augmented progressively with the increase of [TSH] but at >0.10 mU/ml, no further augmentation was observed.

When thyroid cells were cultured in the presence of 1 and 10 ng PGE_2/dish for 5 days (fig.5), no significant uptake and organification of iodine were found. In contrast, when the cells were cultured in the presence of 100, 1000 and 10 000 ng PGE_2/dish, dose-related increases of uptake and organification of iodine were observed; maximal increase was observed at ~1000 ng PGE_2/dish. When thyroid cells were cultured in the presence of graded doses of $PGF_{2\alpha}$, no significant uptake and organification of iodine was observed (fig.5).

4. Discussion

We show here that cultured porcine thyroid cells are able to produce prostacyclin, thromboxane A_2, $PGF_{2\alpha}$ and PGE_2.

When the cells are cultured in the absence of TSH concentrations of PGE_2, $PGF_{2\alpha}$ and thromboxane B_2 in the cells and medium increase progressively for the first 4–5 days of the culture (not shown). When cultured in the absence of TSH for 5 days, the cells produce and release PGE_2, $PGF_{2\alpha}$ and TXB_2. However, [PGE_2] in the cells and medium decreases progressively with increases of [TSH] in the culture medium, as reported [15]. Simultaneously, $PGF_{2\alpha}$ and TXB_2 decrease progressively with the increase of [TSH] in the culture medium.

When the cells are cultured in the absence of TSH, they fail to accumulate iodide and synthesize organic iodine, but when cultured in the presence of TSH, they can take up iodide and organify it; iodide uptake and organic binding of iodine increase progressively with the increase of [TSH] in the culture medium. When cultured in the presence of graded doses of PGE_2, the cells can take up iodide and organify it; iodide uptake and organic binding of iodine increase progressively with the PGE_2 concentrations in the culture medium but $PGF_{2\alpha}$ in the culture medium does not affect iodine uptake or organification.

In the absence of TSH, the cells can produce PGE_2. To test whether this PGE_2 plays some role to maintain iodine metabolism, the thyroid cells were cultured in the presence of graded doses of PGE_2. The [PGE_2] produced by the cells in the absence of TSH were less than those required for the maintainance of iodine metabolism of the cells.

When the thyroid cells are cultured in the absence of TSH for 5 days, PGE_2, $PGF_{2\alpha}$ and TXB_2 contents in the cells and medium are high but when cultured in the presence of TSH, the contents of PGE_2, $PGF_{2\alpha}$

and TXB_2 are extremely low. In contrast to the alterations of PGE_2, $PGF_{2\alpha}$ and TXB_2 contents, when cultured in the presence of TSH, the contents of 6-keto-prostaglandin $F_{1\alpha}$, end-metabolite of prostacyclin, are high but, when cultured in the absence of TSH, the contents of 6-ketoprostaglandin $F_{1\alpha}$ are low. It should be noted that PGE_2, $PGF_{2\alpha}$, TXB_2 and prostacyclin have been proved to be transformed from the unstable precursor prostaglandin H_2. When cultured in the absence of TSH, the cells synthesize PGE_2, $PGF_{2\alpha}$ and TXA_2 but when cultured in the presence of TSH, they synthesize prostacyclin from prostaglandin H_2 and arachidonic acid. It seems that the thyroid cells have enzymes to produce prostacyclin, PGE_2, $PGF_{2\alpha}$ and TXA_2 and that the expression of these enzymes is under the control of TSH. TSH stimulates the synthesis of prostacyclin and inhibits the syntheses of PGE_2, $PGF_{2\alpha}$ and TXA_2 from prostaglandin H_2.

It is noteworthy that as low as 0.1 mU TSH/ml are sufficient to produce maximum augmentation of iodine metabolism and prostacyclin production and maximum inhibitory effects of PGE_2, $PGF_{2\alpha}$ and TXB_2 production. The cells, which maintain their ability to take up iodide and organify it, produce prostacyclin. Prostacyclin strongly stimulates cyclic AMP synthesis and iodine metabolism in the thyroid cells in culture (not shown). Prostacyclin seems to play an important role in the regulation of thyroid function.

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