INCREASES OF PROSTACYCLIN AND PGE_2 LEVELS AFTER ACUTE THYROTROPIN STIMULATION IN CULTURED PORCINE THYROID CELLS

Prostacyclin plays an important role in the presence of thyrotropin

Nobuyuki TAKASU, Seiya SATO, Kazunori TAKAHASHI, Tatsuzo ISHIGAMI, Takashi YAMADA and Yoshifusa SHIMIZU

Department of Medicine, Institute of Adaptation Medicine, Department of Anatomy, Shinshu University School of Medicine, Matsumoto, Nagano-ken 390 and *Kitasato Biochemical Laboratories, Kitasato University Hospital, Asamizodai, Kanagawa-ken 228, Japan*

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1. Introduction

Cultured porcine thyroid cells synthesize prostacyclin (PGI_2) and chronic exposure to thyroid-stimulating hormone (TSH) induces augmentation of PGI_2 synthesis [1]. Following this discovery, PGI_2 was found to be a stimulator of cAMP synthesis and iodine metabolism [2]. However, it is not known whether TSH acutely stimulates PGI_2 synthesis or not. This seems to be very important, since PGI_2 plays a more important role than other prostaglandins (PGs) in the presence of TSH [2] and PGs are postulated to be a modulator of adenylate cyclase–cAMP system [3]. Thus acute effect of TSH on PGI_2 synthesis was studied and it is clearly shown that TSH acutely stimulates PGI_2 synthesis. This offers an idea that PGI_2 could mediate TSH action. In [3,4] TSH was shown to acutely stimulate prostaglandin E_2(PGE_2) synthesis but their results could not be confirmed in [5]. Thus acute effect of TSH on PGE_2 synthesis was also studied and it is concluded that TSH acutely stimulates PGE_2 synthesis.

When cultured in the presence of TSH, thyroid cells preferentially produce PGI_2 and TSH acutely stimulates PGI_2 synthesis. When cultured in the absence of TSH, thyroid cells preferentially produce PGE_2 and TSH acutely stimulates PGE_2 synthesis. In the presence of TSH, PGI_2 plays a more important role than other PGs.

PGE_2 and PGI_2 have been reported to stimulate cAMP synthesis and iodine metabolism [2]. PGI_2 seems to play a more important role than other PGs in the presence of TSH and PGE_2 seems to play a more important role than other PGs in the absence of TSH. However, it is not known whether the cells cultured in the presence of TSH are more sensitive to PGI_2 than PGE_2 or whether the cells cultured in the absence of TSH are more sensitive to PGE_2 than PGI_2. Thus the sensitivities of the cells cultured in the presence or absence of TSH to PGI_2- and PGE_2-stimulations of cAMP synthesis and iodine metabolism were studied.

2. Materials and methods

2.1. *Cell culture*

Thyroid cells were obtained as in [6]. 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 in the absence (control cells) or presence (TSH cells) of 0.1 mM TSH/ml for 6 days.

2.2. *Cell washing*

After 6 days' incubation, cells were centrifuged at $400 \times g$ for 5 min, the supernatants were discarded and the pellets were resuspended in prewarmed (37°C) phosphate-buffered saline (pH 7.4) (PBS) of the following composition (mg/ml): NaCl 8000, KCl 200, $Na_2HPO_4 \cdot 2\,H_2O$ 2890, KH_2PO_4 200, $CaCl_2 \cdot 2\,H_2O$

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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 prewarmed (37°C) phosphate-buffered saline containing 0.1% glucose (PBSG).

2.3. *Prostaglandins measurement*

Aliquots (500 μl) of the washed thyroid cell suspension were preincubated in air for 10 min at 37°C. After this 10 min preincubation, 50 mU TSH/ml dissolved in 1% albumin PBSG solution or 1% albumin PBSG solution was added and the incubation was continued further for the indicated periods and the incubation was terminated by adding 1.5 ml mixture of ethyl acetate, isopropanol and 0.2 N citrate (3:3:1, by vol.) for PGE_2 measurement or by adding 3 ml mixture of chloroform/methanol (2:1, v/v) for 6-keto-prostaglandin $F_{1\alpha}$ (an end-metabolite of PGI_2) measurement and after this termination, the cells were immediately homogenized. PGE_2 and 6 ketoprostaglandin $F_{1\alpha}$ were measured as in [1].

2.4. *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 thyroid-stimulating substances (TSH, PGE_2 or PGI_2). The incubation was ended by immersing the tubes into dry ice–acetone bath until frozen, followed by subsequent immersion into boiling water bath for 2 min. The cells were homogenized and centrifuged. Aliquots of supernatants were diluted appropriately and used for cAMP measurement by radioimmunoassay as in [7].

2.5. *Iodine metabolism*

After 6 days' incubation, iodide uptake and discharge were estimated using washed cell suspension [2]. 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 the indicated periods of incubation in air at 37°C, 5 ml 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 TSH, PGE_2 or PGI_2 was added and then the cell suspensions were incubated further. This incubation was ended by adding 5 ml PBS and washing was performed twice with the same buffer as above. The radioactivity levels of the cell pellets were counted.

The absolute amounts of iodide uptake and discharge were calculated from the specific radioactivities of the original iodide solution.

2.6. *Materials*

Thyroid stimulating hormone (TSH) was obtained from Armour Pharmaceuticals (Phoenix AZ). 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. *Transient increases of PGE_2 and 6 ketoprostaglandin $F_{1\alpha}$ levels after acute TSH stimulation in the thyroid cells cultured in the presence or absence of TSH*

Porcine thyroid cells were cultured in the presence (fig.1B,2B) or absence (fig.1A,2A) of 0.1 mU TSH/ml for 6 days and then thyroid cells were washed with prewarmed PBSG (37°C). The washed thyroid cells were incubated at 37°C in room air for 10 min and then 50 mU TSH/ml was added to see the effects of TSH on the syntheses of PGE_2 (fig.1) and 6 keto-prostaglandin $F_{1\alpha}$, an end-metabolite of prostacyclin (fig.2). TSH acutely stimulated the syntheses of PGE_2 and 6-ketoprostaglandin $F_{1\alpha}$ in control and TSH-supplemented cells, the maximal levels of PGE_2 or 6-keto-prostaglandin $F_{1\alpha}$ being observed at 15 s or 30 s after addition of TSH, respectively.

When cultured in the presence of TSH, the basal concentrations of PGE_2 were extremely low, being 12.5% of those of control cells (fig.1) but the concentrations of 6-ketoprostaglandin $F_{1\alpha}$ were high, being

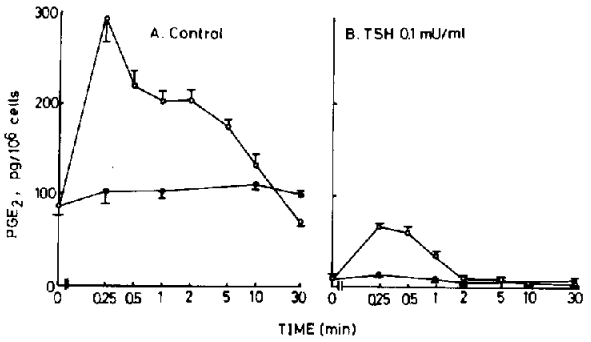

Fig.1. Acute effect of TSH on PGE_2 levels in control (A) and TSH-supplemented (B) cells. Isolated porcine thyroid cells were cultured in the absence (A, control) or in the presence (B, 0.1 mU TSH/ml) of TSH for 6 days and then the thyroid cells were washed with prewarmed PBSG (37°C). The washed thyroid cells were incubated at 37°C in room air for 10 min. After this 10 min preincubation, 50 mU TSH/ml dissolved in albumin solution (○) or the albumin solution containing no TSH (●) was added (0 time) to see the time course of acute TSH effect on PGE_2 synthesis and the incubation was continued further for the indicated periods. Each point is the mean ± SE of triplicate determinations.

150% of those of control cells (fig.2). When cultured in the absence of TSH, PGE_2 concentrations are high.

The magnitudes of increases of PGE_2 and 6-keto-prostaglandin $F_{1\alpha}$ after acute TSH stimulation were also different in control and TSH-supplemented cells;

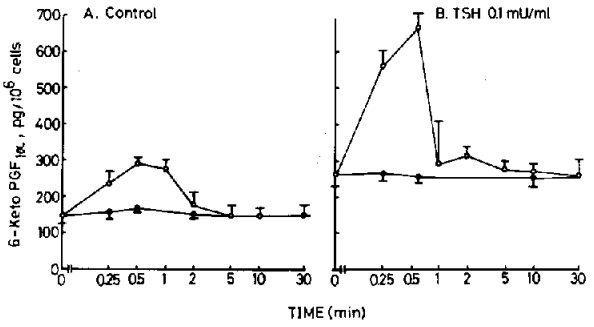

Fig.2. Acute effect of TSH on 6-ketoprostaglandin $F_{1\alpha}$ levels in control (A) and TSH-supplemented (B) cells. Isolated porcine thyroid cells were cultured in the absence (A, control) or in the presence (B, 0.1 mU TSH/ml) of TSH for 6 days and then the thyroid cells were washed with prewarmed PBSG (37°C). The washed thyroid cells were incubated at 37°C in room air for 10 min. After this 10 min preincubation, 50 mU TSH/ml dissolved in albumin solution (○) or the albumin solution containing no TSH (●) was added (0 time) to see the time course of acute TSH effect on 6-ketoprostaglandin $F_{1\alpha}$, an end-metabolite of prostacyclin, synthesis and the incubation was continued further for the indicated periods. Each point is the mean ± SE of triplicate determinations.

when cultured in the presence of TSH, the concentrations of 6-ketoprostaglandin $F_{1\alpha}$ increased up to 670 pg/10^6 cells but when cultured in the absence of TSH, they increased up to 290 pg/10^6 cells, being greatly less than those of the cells cultured in the presence of TSH (fig.2). However, the magnitude of the increase of PGE_2 concentrations after acute TSH stimulation was greater in control cells than that in TSH-supplemented cells; when cultured in the absence of TSH, the PGE_2 concentrations increased up to 295 pg/10^6 cells but when cultured in the presence of TSH, the increase of PGE_2 concentrations was very small, being only up to 67 pg/10^6 cells (fig.1). The increases of PGE_2 and 6 ketoprostaglandin $F_{1\alpha}$ were transient and after attaining their maximal levels, they decreased rapidly toward the basal levels.

3.2. *Acute effects of TSH, PGE_2 and PGI_2 on cAMP synthesis*

Isolated porcine thyroid cells were cultured in the absence (fig.3A) or presence (fig.3B) of 0.1 mU TSH/ml. This concentration (0.1 mU/ml) of TSH maintained the thyroid cells in the best condition and did

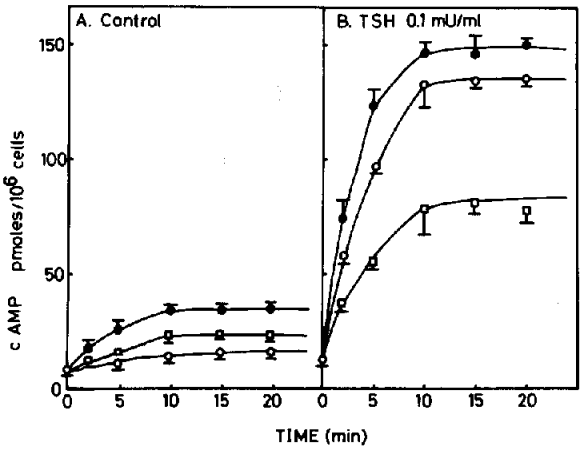

Fig.3. Acute effects of 50 mU TSH/ml (○), 10 μM (3.5 μg/ml) PGE_2 (●) and 10 μM (3.7 μg/ml) PGI_2 (□) on cAMP synthesis in control (A) and TSH-supplemented (B) cells. Isolated thyroid cells were cultured in the absence (A, control) or in the presence (B, 0.1 mU TSH/ml) of TSH for 6 days and then the thyroid cells were washed with prewarmed PBSG (37°C). The washed thyroid cells were incubated at 37°C in room air for 10 min. After this 10 min incubation, 50 mU TSH/ml (○), 10 μM PGE_2 (●) or 10 μM PGI_2 (□) was added (0 time) to see the effects of TSH, PGE_2 and PGI_2 on cAMP synthesis in the presence of 10 mM theophylline and the incubation was continued further for the indicated periods. Each point is the mean ± SE of triplicate determinations.

not induce refractoriness to further stimulation [6–9]. After 6 days' incubation, the cells were washed and then incubated with 50 mU TSH/ml, 10 μM (3.5 μg/ml) PGE_2 or 10 μM (3.7 μg/ml) PGI_2 in the presence of 10 mM theophylline in room air to get the time courses of cAMP synthesis of control and TSH-supplemented cells after acute stimulation with TSH, PGE_2 or PGI_2 (fig.3). In all cases, the maximal responses of cAMP synthesis were obtained after 10 min incubation with thyroid-stimulating substances and the cAMP responses in control cells were always less than those in TSH supplemented cells. In control cells, PGE_2-stimulated cAMP responses were greater than PGI_2- or TSH-stimulated cAMP responses and TSH-stimulated cAMP responses were very small. In TSH cells, PGE_2-stimulated cAMP responses were greater than PGI_2-stimulated cAMP responses. TSH-stimulated cAMP responses were greater than PGI_2-stimulated cAMP responses, being slightly less than PGE_2-stimulated ones.

Acute effects of graded doses of PGE_2 and PGI_2 on cAMP syntheses were studied in thyroid cells cultured in the absence (fig.4A) or presence (fig.4B) of 0.1 mU TSH/ml. After 6 days' incubation in the absence (fig.4A) or presence (fig.4B) of 0.1 mU TSH/ml, the cells were washed and again incubated with graded doses of PGE_2 and PGI_2 in the presence of 10 mM theophylline in room air to get PGE_2- and PGI_2-stimulated dose–response curves of cAMP synthesis in control and TSH-supplemented cells. In both cases, 10 μM PGE_2- and PGI_2-stimulated cAMP syntheses maximally, although 10 μM PGE_2-stimulated cAMP synthesis was >10 μM PGI_2-stimulated one. However, 1–10 nM PGI_2-stimulated cAMP synthesis was higher than 1–10 nM PGE_2-stimulated cAMP synthesis. PGE_2- and PGI_2-stimulated cAMP syntheses in TSH-supplemented cells were always higher than those in control cells.

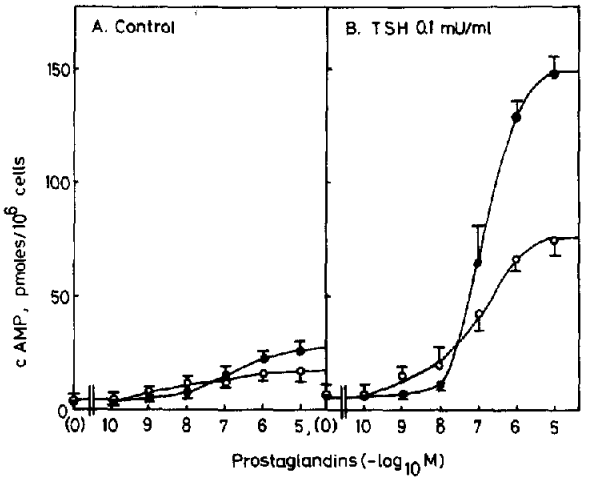

Fig.4. Acute effects of graded doses of PGE_2 (•) or PGI_2 (○) on cAMP synthesis in control (A) and TSH-supplemented (B) cells. Isolated porcine thyroid cells were cultured in the absence (A, control) or presence (B, 0.1 mU TSH/ml) of TSH for 6 days and then thyroid cells were washed. The washed thyroid cells were incubated at 37°C in room air for 10 min. After this 10 min incubation, graded doses of PGE_2 (•) (up to 10 μM (3.5 μg/ml)) or PGI_2 (○) (up to 10 μM (3.7 μg/ml)) were added and the incubations were continued further for 5 min in the presence of 10 mM theophylline to get PGE_2 (•)- and PGI_2 (○)-stimulated dose–response curves of cAMP synthesis. Each point is the mean ± SE or triplicate determinations.

3.3. *Iodide uptake and discharge of accumulated iodide from the thyroid cells by TSH, PGE_2 and PGI_2*

Isolated porcine thyroid cells were cultured in the absence (fig.5A) or presence of 0.1 mU TSH/ml

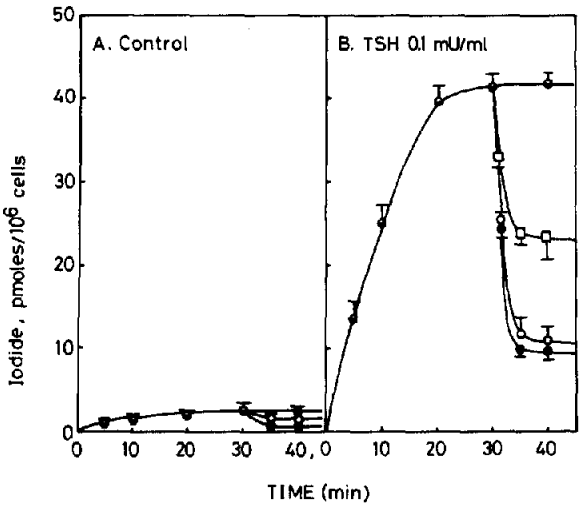

Fig.5. Iodide uptake and acute effects of 50 mU TSH/ml (○), 10 μM (3.5 μg/ml) PGE_2 (•) and 10 μM (3.7 μg/ml) PGI_2 (□) on iodide discharge in control (A) and 0.1 mU/ml TSH-supplemented (B) cells. Isolated porcine thyroid cells were cultured in the absence (A, control) or in the presence (B, 0.1 mU TSH/ml) of TSH for 6 days and then the thyroid cells were washed with prewarmed PBSG (37°C). The washed thyroid cells were incubated with 0.5 μM $Na^{127}I$ and 0.1 μCi $Na^{125}I$ (NaI solution) in the presence of 1 mM MMI. The cells took up iodide (⊛). After 30 min incubation with NaI, 50 mU TSH/ml (○), 10 μM PGE_2 (•), 10 μM PGI_2 (□) or buffer (⊛) was added and then the incubation was continued further for the indicated periods. Each point is the mean ± SE of triplicate determinations.

(fig.5B) for 6 days and then the cells were washed. After washing, the cells were incubated with 0.5 μM $Na^{127}I$ and 0.1 μCi $Na^{125}I$ in the presence of 1 mM MMI and the cells took up iodide. As shown in fig.5, the uptake was maximum at 30 min. After 30 min incubation with iodide, 10 μM (3.7 μg/ml) PGI_2, 10 μM (3.5 μg/ml) PGE_2 or 10 mU TSH/ml was added and these substances caused rapid discharge of intracellular iodide. This iodide discharge was observed within 2 min and the maximum iodide discharge was observed around 5–10 min after adding thyroid stimulating substances. When cultured in the absence of TSH (fig.5A), the thyroid cells took up very small amounts of iodide and TSH-, PGE_2- or PGI_2-stimulated iodide discharge was very small. When cultured in the presence of TSH (fig.5B), the thyroid cells took up large amounts of iodide and significant degrees of iodide discharge were observed after adding thyroid-stimulating substances; TSH- and PGE_2-stimulated iodide discharges are greater than PGI_2-stimulated one and TSH- and PGE_2-stimulated iodide discharges were about equal.

Acute effects of graded doses of PGE_2 and PGI_2 on iodide discharge were studied (fig.6). Thyroid cells were treated as before (fig.5). The differences of iodide concentrations between PGs-added (open symbols) and buffer-added (semi-closed symbols) ones indicated the absolute amounts of iodide discharge induced by added PGs (closed symbols). These absolute amounts of iodide discharge increased gradually and maximum iodide discharge was observed around 5–10 min after adding PGs. The magnitude of this iodide discharge depended on the added PGs-concentrations (fig.6B,C). Maximal iodide discharge was observed when 10 μM PGs were added and the amounts of iodide discharge induced by 10 μM PGI_2 was ~2/3rd of that induced by 10 μM PGE_2. However, 1–10 nM PGI_2-stimulated iodide discharge was

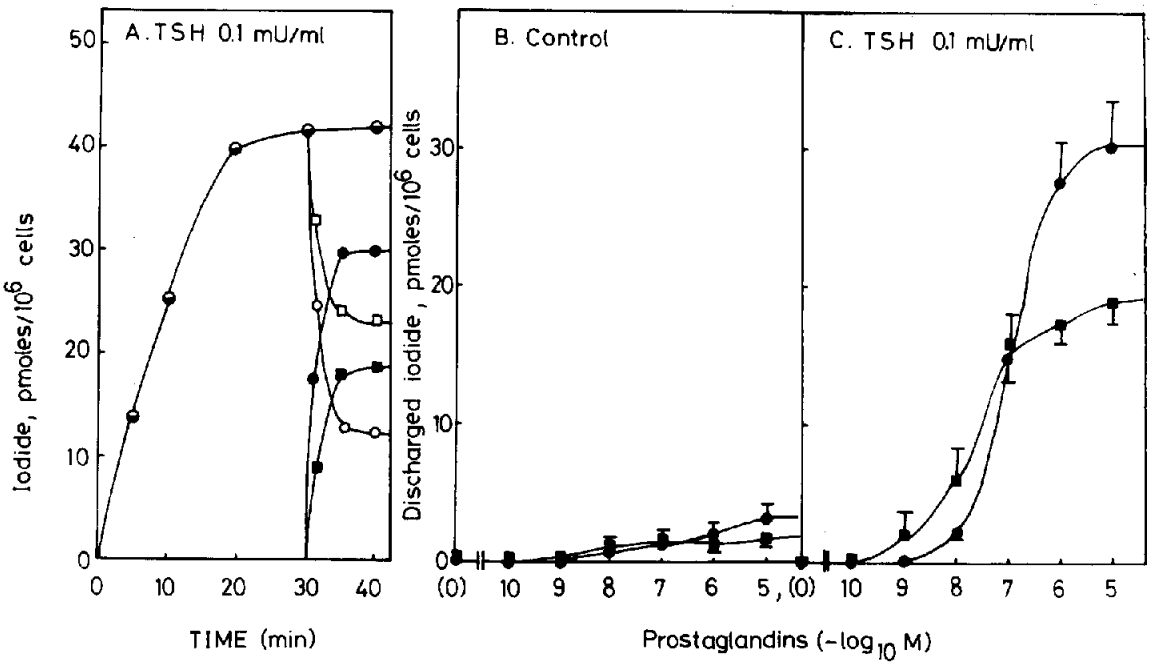

Fig.6. Acute effects of graded doses of PGE_2 or PGI_2 on iodide discharge in control (B) and 0.1 mU/ml TSH-supplemented (A,C) cells. Isolated thyroid cells were cultured and treated as in fig.5. (A) Cells cultured in the presence of 0.1 mU TSH/ml were incubated with NaI solution in the presence of 1 mM MMI. The cells took up iodide (⊖). After 30 min incubation with NaI, 10 μM (3.5 μg/ml) PGE_2 (○), 10 μM (3.7 μg/ml) PGI_2 (□) or buffer (⊖) was added and 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 absolute amounts of iodide discharge (PGE_2 (●); PGI_2 (■)). (B,C) Cells, cultured in the absence (B) or presence (C) of 0.1 mU TSH/ml for 6 days, were washed and then incubated with NaI solution in the presence of 1 mM MMI. After 30 min incubation with NaI, graded doses of PGE_2 (up to 10 μM (3.5 μg/ml)) or PGI_2 (up to 10 μM (3.7 μg/ml)) were added and the incubation was continued further for 10 min to get acute effects of PHs on iodide discharge. The absolute amounts of iodide discharge induced by PGE_2 (●) or PGI_2 (■) were calculated as in (A). Each point is the mean ± SE of triplicate determinations.

higher than 1–10 nM PGE_2-stimulated iodide discharge. PGs-stimulated iodide discharge was always higher in TSH-supplemented cells than in control cells.

4. Discussion

We have shown that cultured porcine thyroid cells produce PGI_2 and PGE_2 and that chronic exposure to TSH induces augmentation of PGI_2 and depression of PGE_2 [1]. Although the precise role of PGs in thyroid physiology is not yet known at present, these paradoxical alterations of PGI_2 and PGE_2 levels will provide some clue to reveal the role of PGs in thyroid physiology. Thus acute effects of TSH on PGI_2 and PGE_2 syntheses were studied in control and TSH-supplemented cells. For comparison, acute effects of TSH, PGI_2 and PGE_2 on cAMP synthesis and iodine metabolism were also studied, since PGI_2 and PGE_2 stimulate cAMP synthesis and iodine metabolism [2]. The sensitivities of control and TSH-supplemented cells to PGI_2- and PGE_2-stimulations of cAMP synthesis and iodine metabolism were compared.

TSH acutely stimulates PGI_2 (measured as an end-metabolite, 6-ketoprostaglandin $F_{1\alpha}$) and PGE_2 syntheses in the cells cultured in the presence or absence of TSH. The increases of PGI_2 and PGE_2 are transient and lasting for several minutes; the maximum increases are observed at 30 s or 15 s and they decline sharply thereafter. The significance and the mechanism for these transient increases of PGs are not known at present. When cultured in the presence of TSH, the basal and TSH-stimulated PGI_2 levels were ~1.5–3-times higher than the PGI_2 levels of the cells cultured in the absence of TSH and the basal and TSH-stimulated PGI_2 levels were ~10-times higher than the PGE_2 levels of the cells cultured in the presence of TSH, indicating that in the presence of TSH, PGI_2 plays an important role. However, when cultured in the absence of TSH, the basal and TSH-stimulated PGE_2 levels were ~5–10-times higher than the PGE_2 levels of the cells cultured in the presence of TSH, indicating that in the absence of TSH, PGE_2 plays an important role. These results extended our idea that, in the presence of TSH, PGI_2 synthetic process is dominant but, in the absence of TSH, PGE_2 synthetic process is dominant [1]. PGI_2 and PGE_2 have been proven to be transformed from the unstable precursor prostaglandin H_2. When cultured in the absence of TSH, the cells synthesize PGE_2 but when cultured in the presence of TSH, they synthesize PGI_2 from prostaglandin H_2 and arachidonic acid. It seems that thyroid cells have enzymes to produce PGI_2 and PGE_2 and that the expression of these enzymes is under the control of TSH. TSH stimulates the synthesis of PGI_2 and inhibits the synthesis of PGE_2 from prostaglandin H_2.

The effects of TSH, PGI_2 and PGE_2 on cAMP synthesis and iodine metabolism were studied in the cells cultured in the absence or presence of TSH. The increases of TSH-, PGI_2- or PGE_2-stimulated cAMP synthesis and iodide discharge in the cells cultured in the presence of TSH are much higher than those in the cells cultured in the absence of TSH. When cultured in the absence of TSH, the amounts of TSH-, PGE_2- or PGI_2-stimulated iodide discharge are very small, indicating that thyroid cells should be maintained in the presence of TSH for the full expression of TSH, PGE_2 and PGI_2 actions. It should also be noted that when cultured in the presence of TSH, the degrees of TSH-stimulated cAMP synthesis or iodide discharge are about equal to those of PGE_2-stimulated cAMP synthesis or iodide discharge, respectively, but when cultured in the absence of TSH, the degrees of TSH-stimulated cAMP synthesis or iodide discharge are greatly less than those of PGE_2- or PGI_2-stimulated ones. The mechanism for this is not known at present.

When cultured in the presence of TSH, the degrees of 10 μM PGI_2-stimulated cAMP synthesis and iodine metabolism are lower than 10 μM PGE_2-stimulated ones but the degrees of 1–10 nM PGI_2-stimulated cAMP synthesis and iodine metabolism are higher than 1–10 nM PGE_2-stimulated ones. The amounts of PGI_2, produced by the thyroid cells in the presence of TSH, are ~1 nM, indicating that, in the physiological ranges of PGI_2 concentrations, PGI_2 plays a more important role than PGE_2.

The importance of prostaglandins (PGs) in the thyroid physiology has been a controversial subject for many years but it is generally acknowledged that:

(1) PGE compounds stimulate thyroid adenylate cyclase and cAMP synthesis [10,11] and iodine metabolism [10] and reproduce some of the effects of TSH in vitro [10] and PGI_2 has similar effects on cAMP synthesis and iodine metabolism [2];

(2) TSH stimulates PGI_2 and PGE_2 syntheses [3].

There have been proposed several hypotheses to connect these two, intending to find the role of PGs in

the thyroid receptor–adenylate cyclase and cAMP system. It has been suggested that PGs could mediate the stimulatory effect of TSH on thyroid adenylate cyclase [12]. However, it was later shown that the mechanism of activation of adenylate cyclase by TSH and PGE compounds are different [13–16] and it is also reported that prostaglandin synthesis is not an obligatory step in the activation of thyroid functions by TSH [17]. So far, the efforts to connect these two are unsuccessful.

When cultured in the absence of TSH, the increase of TSH-stimulated PGE_2-synthesis is extremely high but the increases of TSH-stimulated cAMP synthesis and iodine metabolism are negligible. This suggests that the response of PGE_2 synthesis and the responses of cAMP synthesis and iodine metabolism after TSH stimulation are regulated differently and independently. When cultured in the presence of TSH, acute effects of TSH on PGI_2 synthesis, cAMP synthesis and iodine metabolism are clearly observed, proposing several ideas for the roles of PGI_2:

(1) TSH augments cAMP levels and iodine metabolism through PGI_2 production;
(2) TSH augments PGI_2 levels through cAMP production;
(3) The processes to increase PGI_2 levels and to stimulate cAMP synthesis and iodine metabolism are independent.

The TSH-stimulated PGI_2 increases are giving some information to the cells, the nature of this information being unknown at present. Further studies are required to reveal the role of PGI_2 in thyroid physiology.

In [3] TSH increased PG synthesis, especially PGEs, in isolated bovine thyroid cells but this observation was not confirmed in [5,18]. In [1,18] the depressive effect of TSH on PGE_2 synthesis was reported. Although equally using isolated thyroid cells, the results obtained by Burke et al. and Margotat et al. and us were quite different and in [19] it was stated that it is difficult to speculate on the reason for the discrepancies between them. However, this communication gives a possible explanation for these discrepancies. The chronic and acute effects of TSH on PGE_2 synthesis are completely different; TSH acutely stimulates PGE_2 synthesis, which was originally observed in [3] and confirmed by us here and TSH chronically depresses PGE_2 synthesis, which was originally reported in [18] and confirmed by us in [1]. The exact mechanism for these differences of the acute and chronic effects of TSH on PGE_2 synthesis is not yet known and further studies are required.

When cultured in the presence of TSH, PGI_2 synthetic process is dominant and when cultured in the absence of TSH, PGE_2 synthetic process is dominant. TSH reveals the expression of the enzyme that synthesizes prostacyclin from prostaglandin H_2 or arachidonic acid and the mechanism for this expression needs to be clarified. When cultured in the presence of TSH, thyroid cells preferentially produce PGI_2 and TSH acutely stimulates PGI_2 synthesis and when cultured in the absence of TSH, thyroid cells preferentially produce PGE_2 and TSH acutely stimulates PGE_2 synthesis. In the presence of TSH, PGI_2 plays more a important role than other PGs.

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